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Social and Developmental Biology of the Myxobacteria

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INTRODUCTION

The myxobacteria are distinguished from other bacteria by their remarkable social behaviors, most notably the formation of multicellular fruiting bodies containing dormant myxospores. Many of these behaviors were recorded 25 years ago in a series of films by Reichenbach and colleagues that have inspired much of the research described in this article (198, 202, 204–207). Continued research in this area will undoubtedly provide insights into the molecular mechanisms of these behaviors. Most studies on the biology of social behavior have been limited to one species, *Myxococcus xanthus*, which has an excellent genetic system and is amenable to a variety of experimental approaches. Several studies on *Stigmatella aurantiaca* suggest that it is a complementary system worthy of continued investigation. This article will focus on the social and developmental biology of the myxobacteria, primarily these two species. Information concerning aspects of myxobacterial research not covered in this article may be found in a book (215) and in several review articles (47, 50, 110, 111, 158, 199, 201, 229, 230, 270, 284; D. R. Zusman, *in* M. Inouye, J. Campisi, D. Cunningham, and M. Riley, ed., *Gene Expression and Regulation of Cell Growth and Development*, in press; D. R. Zusman, M. J. McBride, W. R. McCleary, and K. A. O'Connor, Symp. Soc. Gen. Microbiol., in press; B. C. Lampson, S. Inouye, and M. Inouye, submitted for publication).

The resistance of myxospores to desiccation and freezing enables myxobacteria to survive seasonally harsh environments such as arid deserts and arctic tundra and may have been the major factor contributing to their worldwide distribution. Although myxobacteria are a common and perhaps even significant component of many aerated soil ecosystems, it has been difficult to determine their actual numbers because of their slow growth, the lack of a selective medium, and the propensity of the cells to stick to one another. Neutral or slightly alkaline soils can contain as many as 450,000 cells per g (157, 238), and decomposing compost contains more than 500,000 cells per g (238). Despite the prevalence of myxobacteria in soil systems, virtually nothing is known of their contribution to soil ecology. Myxobacteria are microbial predators, and it is likely that they play a role in controlling the population size of certain soil microorganisms.

Myxobacteria contain a vast reservoir of antibiotics with unusual chemical structures (203, 217). Between 60 and 80% of more than 800 wild isolates produce antibiotics with chemical structures that have not been described before (203) (Fig. 1). Several of these antibiotics may prove to be clinically useful. Sorangicin inhibits transcription initiation in eubacteria and shows some promise as a broad-spectrum antibacterial compound (102). Ambruticin is an antifungal antibiotic (211) that is effective in the treatment of systemic mycoses (137).

TAXONOMY AND LIFE CYCLE

All myxobacterial species appear to be related on the basis of their DNA base composition, which is 67 to 71 mol% G+C (145, 159), and 16S rRNA oligonucleotide cataloging (140). Comparison of the 16S rRNA molecules from a variety of myxobacteria indicate that they are a distinct taxonomic

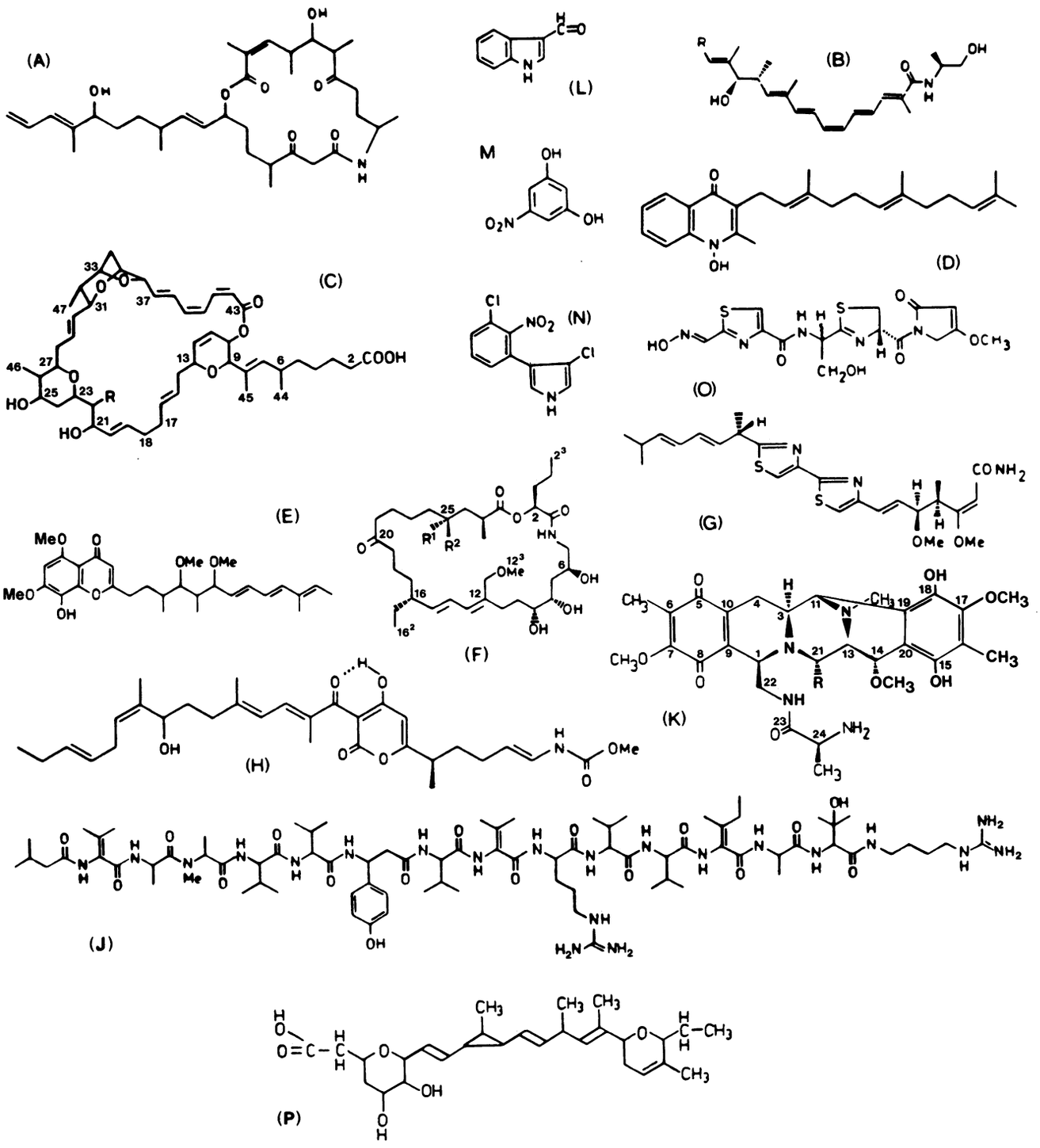


FIG. 1. Antibiotics isolated from the myxobacteria (203). (A) Angiolam A; (B) myxalamid; (C) sorangicin A; (D) aurachin C; (E) stigmatellin A; (F) myxovirescin; (G) myxothiazol A; (H) corallopyronin A; (J) myxovalargin; (K) saframycin Mx1; (L) 3-formylindole; (M) 5-nitroresorcinol; (N) pyrrolnitrin (previously identified in *Pseudomonas* spp.); (O) althiomycin (previously identified in *Streptomyces* spp.); (P) ambruticin.

group that is more closely related to the bdellovibrios and the sulfur- and sulfate-reducing bacteria than to other groups of gliding bacteria (60, 187, 276). On the basis of the 16S rRNA data it has been suggested that the myxobacteria and bdellovibrios are aerobic descendents of an ancestral sulfur-metabolizing bacterium (276).

There is considerable disagreement about the nomenclature used in the taxonomy of the myxobacteria. Assigning phylogenetic relationships is complicated by the fact that type materials are sometimes represented by herbarium specimens of fruiting bodies, which provide little information about the overall phenotype and genotype of the cell.

TABLE 1. Taxonomy of the myxobacteria

Taxonomy in *The Prokaryotes* (201)	Taxonomy in *Bergey's Manual* (158)
Order *Myxobacterales*	Order *Myxococcales*
Suborder *Cystobacterineae*	Family I. *Myxococcaceae*
Family I. *Myxococcaceae*	Genus *Myxococcus*
Genus *Myxococcus*	Family II. *Archangiaceae*
Genus *Corallococcus*	Genus *Archangium*
Genus *Angiococcus*	Family III. *Cystobacteraceae*
Family II. *Archangiaceae*	Genus I. *Cystobacter*
Genus *Archangium*	Genus II. *Melittangium*
Family III. *Cystobacteraceae*	Genus III. *Stigmatella*
Genus *Cystobacter*	Family IV. *Polyangiaceae*
Genus *Melittangium*	Genus I. *Polyangium*
Genus *Stigmatella*	Genus II. *Nannocystis*
Suborder *Sorangineae*	Genus III. *Chondromyces*
Family *Sorangiaceae*	
Genus *Sorangium*	
Genus *Polyangium*	
Genus *Haploangium*	
Genus *Chondromyces*	
Genus *Nannocystis*	

The establishment of a collection of type strains that can be subjected to rigorous molecular analysis will undoubtedly help resolve the taxonomy of these organisms (64).

The two most recent versions of myxobacterial taxonomy are summarized in Table 1 (158, 201). Myxobacteria have been divided into two different groups based upon simple morphological examination of the cells, spores, and colonies, and this division is consistent with the 16S rRNA catalog data (140). Members of the families *Myxococcaceae*, *Archangiaceae*, and *Cystobacteraceae* have long, slender, rod-shaped vegetative cells with tapered ends that change their shape considerably during myxospore formation (Fig. 2). Their colonies absorb the diazo dye Congo red and tend to be brightly pigmented with monocyclic carotenoids containing reddish keto pigments (208). Members of these families also form ridges of cells known as ripples, which move processively in waves. The similarity of the 16S rRNA oligonucleotide catalogs of these three families suggests that they could be combined into a single genus despite large differences in the structures of the fruiting bodies (140). Nucleic acid homology and sequencing studies should provide a more sensitive indication of the relatedness of these families (106, 267). Members of the other family, *Sorangiaceae* (158), also called *Polyangiaceae* (201), have short rod-shaped vegetative cells with blunt ends that change their shape little upon myxospore formation (Fig. 2). Colonies of members of this family do not adsorb Congo red or move in ripples. The carotenoids lack the keto pigments and, as a result, are generally more yellowish than those produced by the other families. The 16S catalog data suggest that this family is much more distantly related to the others. The antibiotics isolated from this family do not overlap those of the other families, again pointing to distinct phylogenetic differences between the two groups (203). Scanning electron micrographs of fruiting bodies from several species are shown in Fig. 3; they demonstrate the range of fruiting-body morphologies observed. Within each of the two myxobacterial groups the fruiting-body morphology varies from raised mounds of spores to elaborate treelike structures.

The life cycle of *M. xanthus* is illustrated in Fig. 4 (for scanning electron micrographs depicting the various temporal stages of fruiting-body formation, see Fig. 8). In the presence of nutrients the cells grow and divide indefinitely by transverse binary fission. Fruiting-body morphogenesis is initiated by nutrient deprivation, and tens of thousands of cells direct their movement toward a discrete aggregation focus where they pile atop one another to form a raised mound. Within this mound the rod-shaped cells begin to shorten and assume a spherical shape (Fig. 2). The myxospores are dormant, refractile, and resistant to a variety of environmental insults, such as heat, desiccation, and freezing, that would kill vegetative cells (248). Myxospores germinate when a nutrient source becomes available; they do this by shedding the spore coat and elongating into a rod-shaped cell. Some of the developmental events associated with aggregation and fruiting-body formation can be bypassed with chemical inducers or mutations that stimulate cells to convert directly to myxospores. Secondary alcohols such as glycerol induce myxospore formation in about 90 min, compared with 48 h during fruiting-body morphogenesis (49). A dominant mutation known as *csp-500* has been described that results in sporulation upon temperature shift down to 15°C and bypasses early developmental steps (209).

The primary difference in the life cycle of *S. aurantiaca* is the morphological complexity of the fruiting body. Following aggregation of cells into a raised mound, an elaborate treelike structure is formed. The myxospores are contained in saclike structures, referred to as sporangioles (158) or sporangia (201), that are borne on top of a large stalk. Sporulation can be induced in the absence of fruiting-body formation by treatment of cells with glycerol or a variety of monovalent cations, especially Li^+, NH_4^+, and Na^+ (200).

SOCIAL AND DEVELOPMENTAL BEHAVIORS

On studying the Reichenbach films it becomes clear that the behavior of cells in myxobacterial colonies is synchronized by cell-cell interactions. The premise of the studies described in this review is that this social behavior is governed by rules that can be discerned at the molecular level by a combination of behavioral, genetic, and biochemical analyses. Indeed, the basic genetic structure of each of the behavioral systems has been established, and the details of how these behavioral systems operate are proving to be amenable to conventional experimental approaches.

Myxobacteria display a wide range of social adaptations that are used in feeding, movement, and development. These social behaviors involve thousands of cells and are dependent on three interrelated properties of the cells: the secretion of many types of molecules, gliding motility, and cell-cell contact-mediated interactions. Myxobacteria release a wide variety of molecules into the environment; these include antibiotics (203, 217), peptidoglycan components (234, 235), glycopeptides (52), proteins (249), polysaccharides (73, 74, 251), purines and pyrimidines (232), and siderophores (128). In fact, the cells are usually embedded in an extracellular matrix that is often inappropriately referred to as slime (even though it is not slimy). The mechanisms by which these molecules are released into the extracellular matrix are poorly understood and are currently under investigation. Several mutations have been reported that impair the release of proteins (13, 174). Preliminary investigations seem to favor the existence of specific secretory pathways. Mutations that decrease the extracellular proteolytic activity do not decrease the level of extracellular polysaccharide or that of the glycopeptide myxaline (152).

Compared with flagellar motility, myxobacterial gliding is a relatively slow form of movement that requires a solid surface. Gliding cells often move in large, organized groups

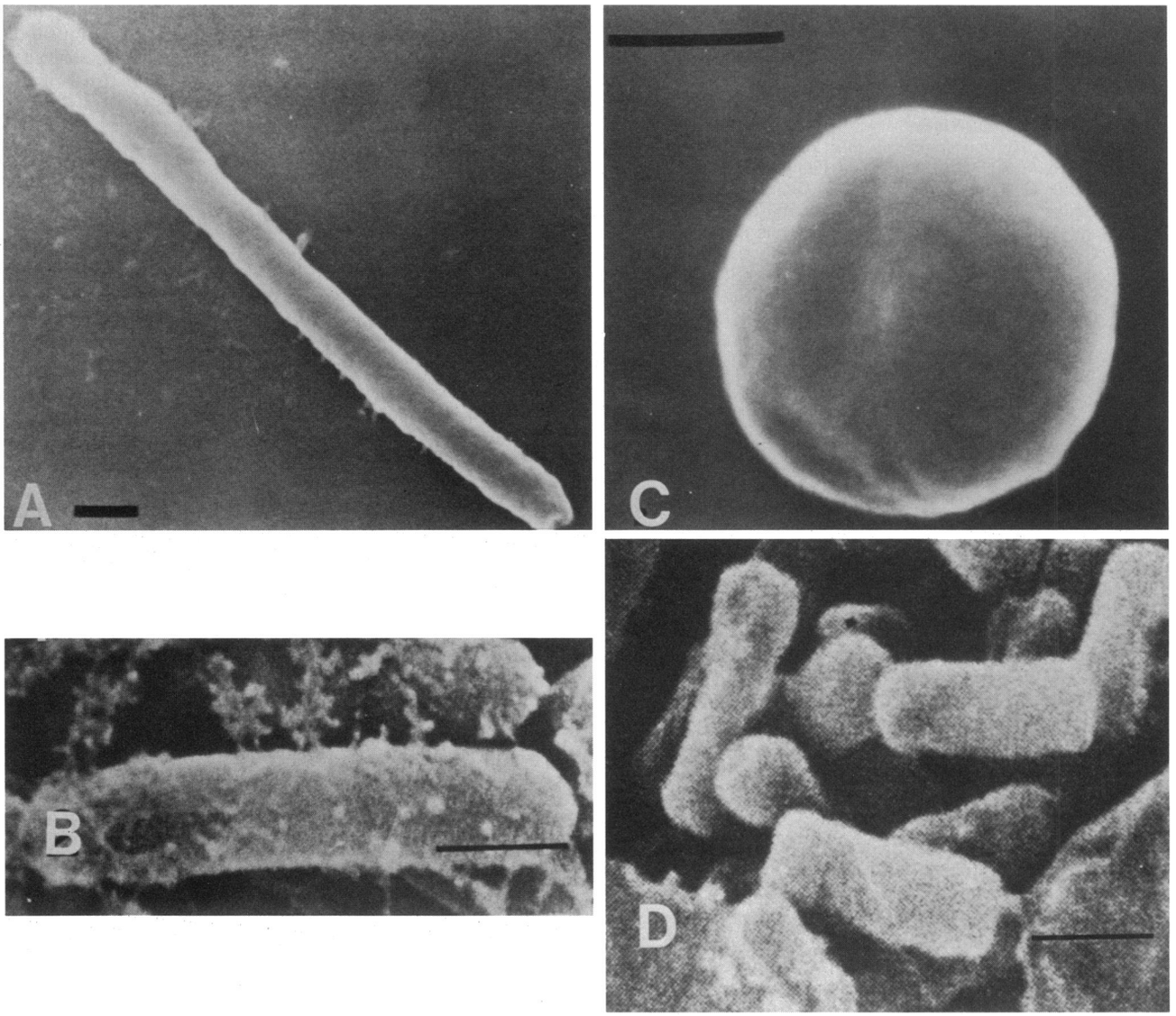


FIG. 2. Scanning electron micrographs of myxobacterial cells and spores. (A) *M. xanthus* vegetative cell (237) (bar, 1 μm). (B) *Polyangium cellulosum* vegetative cell (132) (bar, 1 μm). (C) *M. xanthus* myxospore (237) (bar, 0.5 μm). (D) *Polyangium cellulosum* myxospore (132) (bar, 1 μm). (Reprinted with permission.)

known as swarms, whose members maintain continuous contact with adjacent cells during movement. Because of the proximity of the cells, molecules released by one cell can benefit both the producer and its neighbors. The production of a communal extracellular matrix is characteristic of all myxobacteria, and the structure of the extracellular matrix may play an essential role in several social behaviors. Cell-cell contact is also an important element of myxobacterial social behavior. In fact, gliding cells moving in opposite directions frequently stop and rub vigorously against one another as if specific recognition mechanisms existed. Secretion, gliding motility, and cell-cell contact-mediated interactions are common themes in the five types of social behaviors described below.

Two Multigene Systems Govern Gliding-Cell Behavior

Gliding cells move in the direction of the long axis of the cell, with occasional stops and reversals of direction. There is no obvious organelle providing the propulsion, and there are many conjectures about the mechanism of gliding (for a review, see reference 17), but no single model has gained general acceptance. Since gliding bacteria are widely distributed taxonomically among the eubacteria (276), gliding motility may have several independent origins and consequently several distinct mechanisms. However, the close taxonomic relatedness of the myxobacteria makes it likely that all myxobacteria move by the same mechanism.

Myxobacterial gliding is communal in that cells often move in groups while maintaining contact with other cells. An elegant genetic study by Hodgkin and Kaiser has revealed the framework for the organization of the gliding behavioral hierarchies in *M. xanthus*. Gliding-cell behavior is controlled by two nearly separate multigene systems known as A and S (87, 88). A^+S^+ cells glide individually or in groups ranging in size from several cells to thousands of cells (Fig. 5). Cells with a single mutation in the A system

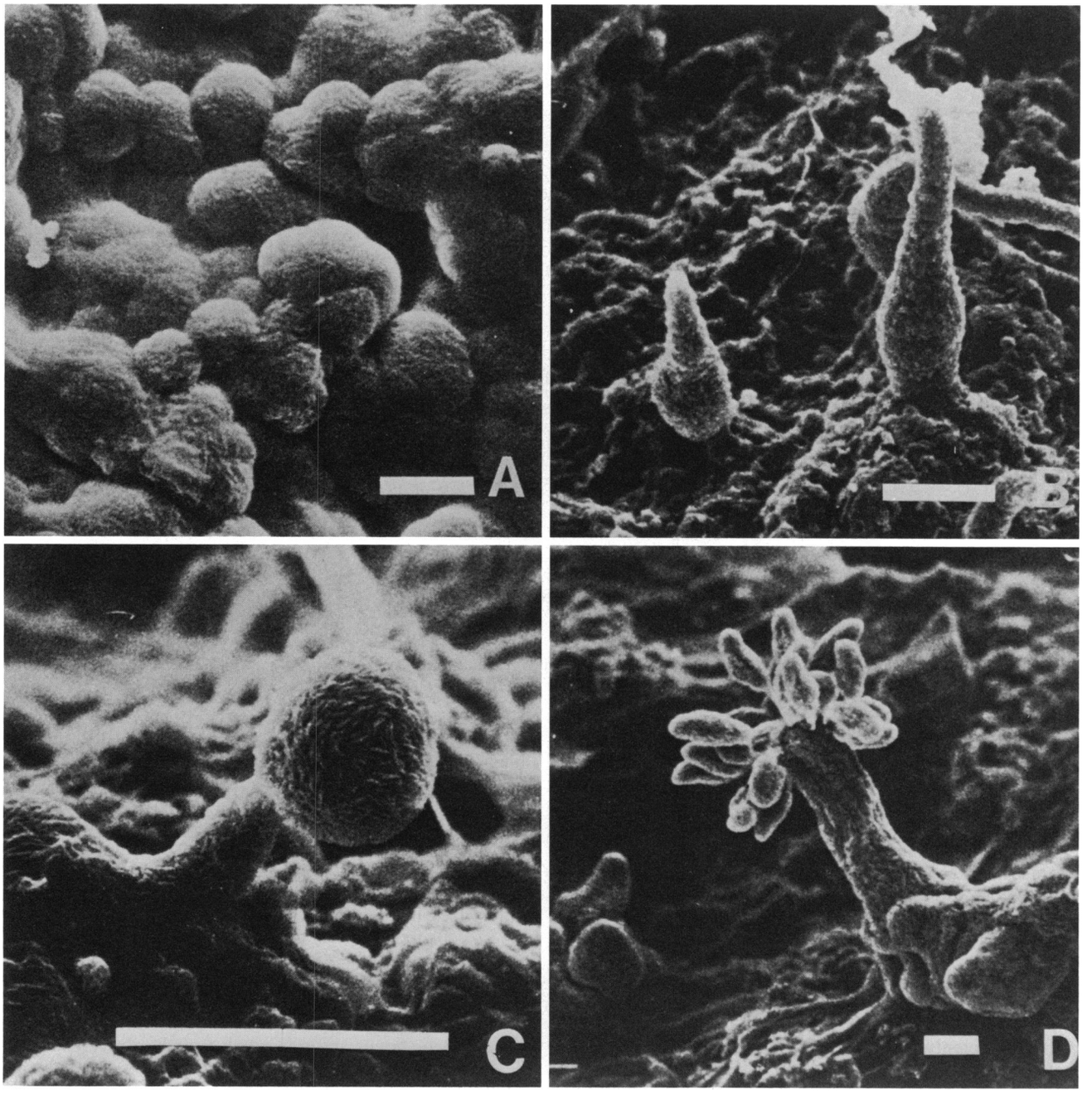


FIG. 3. Scanning electron micrographs of myxobacterial fruiting bodies indicating the morphological diversity (15). (A) *Polyangium cellulosum*. (B) *Myxococcus* (*Corallococcus*) *coralloides*. (C) *Melittangium lichenicolum*. (D) *Stigmatella aurantiaca*. Bar, 20 μm. (Reprinted with permission.)

(A^-S^+) have lost the adventurous aspect of their behavior and can move only when near another cell, as if cell contact is necessary to engage S motility (112). Conversely, cells with a single mutation in the S system (A^+S^-) have lost the social aspect of their motility and glide primarily as individuals or in long thin flares. Cells become nonmotile when they acquire a mutation in the S system plus a mutation in the A system (A^-S^-). Cells also become nonmotile when they acquire a mutation in the *mgl* locus.

Since mutations in the *mgl* locus are the only known mutations that can make a cell nonmotile in a single step, this locus must be required for both A and S motility. The *mgl* locus contains two putative protein-coding regions (244, 246). The DNA sequence of seven *mgl* mutants was compared with that of the wild type and found to differ by a single base pair change in each case (244). Six of the mutations are located in the downstream open reading frame, and this gene has been designated *mglA*. The upstream open reading frame contains the remaining mutation. The cellular location and function of MglA is likely to reveal much about the mechanism of gliding. Recently, a remarkably intricate structure composed of filaments and rings was

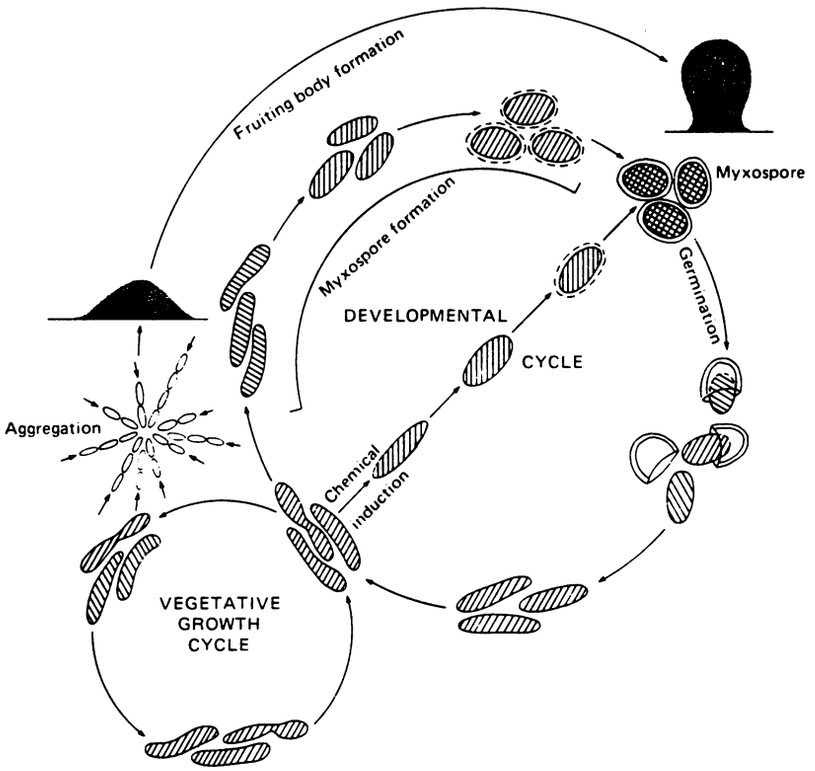


FIG. 4. Life cycle of *M. xanthus*. (Reprinted from reference 47 with permission.)

observed in membrane preparations of *Myxococcus fulvus* and proposed to be the gliding apparatus (141). The effect of *mgl* mutations on the assembly of this structure may help test this notion.

Adventurous motility. The adventurous motility system contains at least 22 loci, *aglA* through *aglH*, *aglJ* through *aglR*, and *cglB* through *cglF* (86, 87, 241). Each of the *cgl* mutants may be stimulated to move upon contact with adjacent cells of a different genotype, whereas *agl* mutants cannot be stimulated to move. Motility is not required to be a stimulation donor. For example, any *cglB*$^+$ strain can stimulate any *cglB* strain regardless of whether the *cglB*$^+$ donor is nonmotile as a result of mutations at other motility loci. The *cglB* recipient temporarily becomes *cglB*$^+$, but its motility depends on the rest of its genotype. For example, a *cglB* S$^-$ recipient will become A$^+$ but remain S$^-$. Contact-stimulated motility is transient in that stimulation decays with time once contact has been broken. The high efficiency of contact stimulation suggests that the particular proteins involved are readily accessible to adjacent cells. Furthermore, many insertions of Tn*phoA* that secrete alkaline phosphatase appear to be fused with *cgl* genes, suggesting that the Cgl proteins are exported (M. Kalos and J. Zissler, Proc. Natl. Acad. Sci. USA, in press).

The O antigen of lipopolysaccharide (LPS) seems to be involved in adventurous motility. A series of monoclonal antibodies (MAbs) were isolated which bind to LPS (65, 66). The reactive epitope was identified as either core or O antigen by probing LPS molecules separated in polyacrylamide gels (58). Tn*5* mutagenesis was used to isolate mutants that failed to react with anti-LPS MAbs (57). One group of mutants was defective in the synthesis of an epitope on the O antigen as well as adventurous motility (59). Other mutations affecting synthesis of epitopes on the core did not eliminate A motility. Mutations in 17 other *agl* loci did not inhibit reactivity with MAbs directed against LPS, and the functions of these loci are unknown.

The contribution of the adventurous motility system to the behavior of wild-type cells has been examined by comparing the phenotypes of A$^+$S$^+$, A$^-$S$^+$, and A$^+$S$^-$ cells. The swarm expansion assay measures the rate at which cells colonize new areas. The expansion rate of all three cell types increases with the cell density, although there are noticeable differences in the shapes of the plots (112). The plot for A$^+$S$^+$ cells more closely resembles that of A$^+$S$^-$ cells than A$^-$S$^+$ cells, implying that adventurous motility is the primary means used by wild-type cells to colonize new areas. Time-lapse videomicroscopy has been used to observe the taxis of *M. xanthus* toward colonies of the prey bacterium *Micrococcus luteus* and toward glass and polystyrene latex beads of similar size (46). A$^+$S$^+$ and A$^+$S$^-$ cells seem to show direct movement to all three types of objects, while A$^-$S$^+$ cells do not. The mechanism directing cell movement is unlikely to involve chemotaxis (48). One possible mechanism is elasticotaxis, in which myxobacteria orient their movement along lines of stress in the agar (243). The location of the LPS O antigen on the exterior of the cell would enable it to interact with the substratum on which the cell moves.

Social motility. The S system contains at least 10 loci, *sglA* to *sglH*, *tgl* (86, 88), and *dsp* (228). Among the S-system mutants, only the *tgl* mutants may be stimulated to regain S motility by contact with *tgl*$^+$ cells (86, 88). A functional S system appears to be necessary for social behaviors such as fruiting-body formation (88) and rippling (234). Cell-cell contact is essential for S motility since A$^-$S$^+$ cells that are separated by more than one cell length are nonmotile (16, 112). When cells are in contact with each other, the rate of A$^-$S$^+$ cell movement away from a colony increases with the

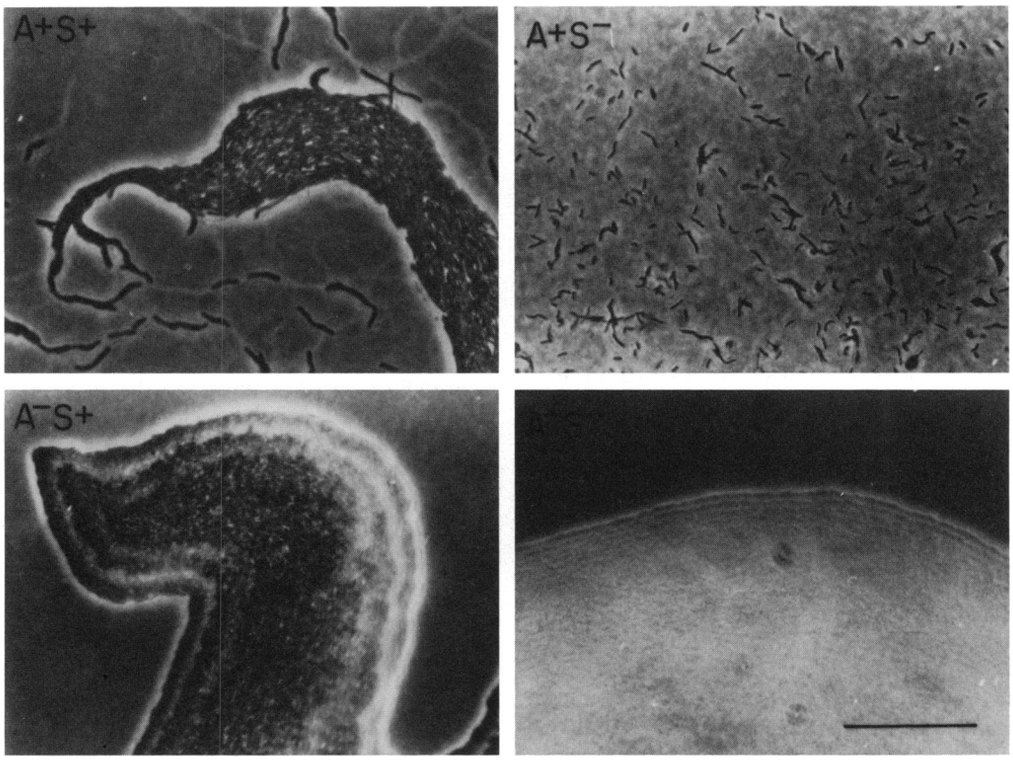


FIG. 5. Effect of A (adventurous) and S (social) motility mutations on *M. xanthus* cell behavior (227). Bar, 50 μm. (Reprinted with permission.)

cell density over a broad range of densities (112). These results suggest that intercellular contact is necessary to engage the motility apparatus and that specific cell-cell recognition systems are likely to exist. Cell surfaces contain two types of appendages that are capable of spanning short distances to interact with nearby cells, pili and fibrils (Fig. 6). Pili are located on one of the cell poles and are about 5 nm wide and one cell length long (40, 109, 142). All S^- mutants, with the exception of *dsp*, are unable to produce pili (3, 109). Pilus production can be induced in *tgl* cells when they are brought into contact with tgl^+ cells and transiently stimulated to regain S motility (109). Fibrils are about 50 nm wide and extend away from the cell into the extracellular space (3). In older colonies and in developing cells a substantial extracellular matrix is formed, possibly by coalescence or crystallization of the fibrils. The neutral polysaccharide fraction secreted by *M. xanthus* amounts to 5 to 10% of the total dry weight of the cells (251). Many S mutants fail to produce a glycocalyx and extracellular matrix (2, 3).

One assay for S-dependent cell-cell interactions is the agglutination assay in which dispersed cells attach to each other, forming large clumps that settle out of suspension (72,

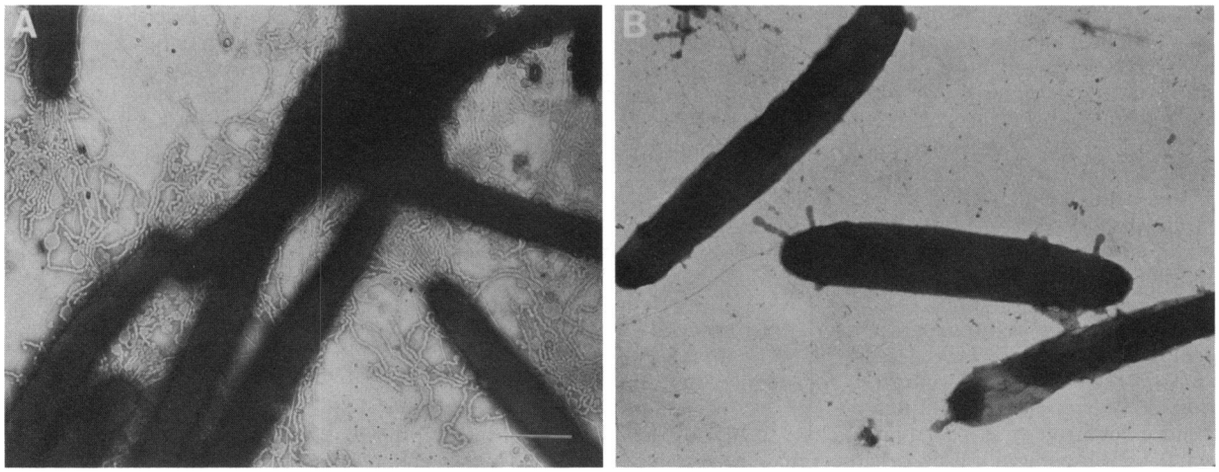


FIG. 6. Transmission electron micrograph of negatively stained wild-type (A) and *dsp* (B) *M. xanthus* cells (3). Note the fibrils associated with the cells in panel A and the pili associated with the cell poles in panel B. Bars, 1 μm. (Reprinted with permission.)

194, 228). There is both a high-affinity binding system, which may be species specific, and a low-affinity binding system, which may be used to attach to prey bacteria (194). In *S. aurantiaca* and *Stigmatella erecta* there are at least two shear-resistant cohesion systems (72). The class A cohesion system is constitutively expressed and energy independent. Class B cohesion is induced by Ca^{2+} and inhibited by respiratory poisons. Ca^{2+} is also required for the induction of gliding motility as well as gliding itself in *S. aurantiaca* (277).

In *M. xanthus*, cell agglutination requires the divalent cations Mg^{2+} and Ca^{2+}, an energy source, a functional S system (228), and the *asgA*, *asgB*, and *asgC* loci (129). Cohesion is energy dependent and is inhibited by cyanide and azide, which block electron transport, carbonyl cyanide-4-(trifluoromethoxy)phenylhydrozone (FCCP), which uncouples oxidative phosphorylation, and *N,N'*-dicyclohexylcarbodiimide (DCCD), which prevents ATP synthesis by the membrane-bound ATPase. The source of the energy was not determined because it is difficult to distinguish between a proton motive force requirement and a nucleoside triphosphate requirement since dissipation of the protein motive force results in ATP hydrolysis in a futile effort to restore the membrane potential (72). Energy may be required for the synthesis or secretion of cell surface components necessary for agglutination. In *Escherichia coli*, protein export requires a proton motive force and is specifically inhibited by ethanol (6, 34, 53). Similar concentrations of ethanol also block agglutination of *M. xanthus* (228). Mutations in the *sgl*, *asg*, and *tgl* loci reduce cohesion at least fivefold, whereas mutations in the *dsp* locus eliminate cohesion altogether (129, 228).

Pili and fibrils are the leading candidates for the cohesin(s) and the S-dependent cell-cell recognition system because of their location on the cell surface and their correlation with S motility. Within minutes after induction of agglutination, large quantities of fibrils appear on the cell surface (3). Treatment of wild-type *M. xanthus* cells with Congo red inhibits agglutination and fibril formation (2, 3). Removal of the cell-associated Congo red restores fibril production and agglutination. Wild-type cells bind Congo red with a K_a of $2.4 \times 10^5\ M^{-1}$ and have about 4.5×10^6 binding sites per cell (2). The *dsp* mutants lack this Congo red receptor. Fibrils of similar size appear to mediate cohesion in a number of diverse bacterial species including *Zoogloea ramigera* (51), *Rhizobium leguminosarum* (239), and *Acetobacter xylinum* (78). The chemical composition of the Congo red receptor is unknown, as is the mechanism by which Congo red inhibits fibril formation and agglutination. Congo red binds certain proteins and oligosaccharides with a high degree of specificity (278). The dye-ligand interaction frequently changes the physical properties of polysaccharide receptors. Congo red inhibits cellulose microfibril formation in *A. xylinum* by preventing the crystallization of the polymerized glycans, keeping them in a soluble form (29). It also inhibits chitin microfibril assembly in the algae *Poterioochromonas* spp. (85) and *Acanthosphaera* spp. (222).

Although the Congo red receptor appears to be necessary for agglutination, other factors also appear to be involved. Mutations in the *asgA*, *asgB*, and *asgC* loci reduce, but do not completely eliminate, agglutination (129). All three mutants bind Congo red with a K_a that is close to that of the wild type. Developing wild-type cells secrete a heat-stable factor that partially restores cohesion to both an *asgB* mutant and an *sglA* mutant. The chemical nature of this factor is unknown. These results suggest that several factors may be involved in cohesion.

A^+S^+ cells can venture away from a colony by using the A motility system and do so more rapidly at high than at low densities (2, 112). Cell density also appears to regulate cell cohesiveness, which is measured in terms of collision efficiency or the average number of collisions required for cell cohesion. The collision efficiency decreases with increasing cell density (3). These results suggest that adventurous behavior might result from reduced synthesis or activity of the cohesin(s).

Other loci controlling gliding-cell behavior. Mutations in other genetic loci that are neither A- nor S-system components also interfere with motility (59, 228, 283). The most carefully studied of these are the *frz* mutations (283). The wild-type locus has been cloned (10), and six genes, *frzA*, *frzB*, *frzCD*, *frzE*, *frzF*, and *frzG*, were discovered by genotypic complementation and DNA sequence analysis. Although two different phenotypes were observed with *frzCD* mutations, a single gene product was observed in *E. coli* maxicells (12) and DNA sequencing revealed a single gene (154).

The *frz* genes control the frequency with which myxobacterial cells reverse their direction of movement (11). Wild-type cells reverse their direction of movement every 6.8 min on the average, but net movement is accomplished because of the large variations in the interval between switching. The majority of the Frz mutants reverse their direction of movement every 2 h, and as a result the cells tend to glide in long streams. Some FrzCD mutants reverse their direction of movement every 2.2 min, but there is little variation in the interval between reversals and, as a result, the cells show little net movement.

Substantial amino acid similarity is observed between the Frz proteins and proteins that control directional movement in enteric bacteria. FrzCD has 40% amino acid homology with the *Salmonella typhimurium* Tar protein over about one-third the length of the protein (154). The Tar protein is a methyl-accepting chemotaxis protein, involved in chemoreception and adaptation, that controls the frequency of runs and tumbles by controlling the frequency of change in the direction of flagellar rotation. Like Tar, FrzCD is modified by methylation probably at glutamate residues since the methyl groups are base labile (156). FrzF probably encodes the methyltransferase, since methylation of FrzCD does not occur in *frzF* cells. FrzA contains 28% amino acid homology with CheW from *S. typhimurium*, which is a protein that controls the clockwise rotation of the flagellar motor (154). FrzE is homologous to both CheA and CheY of *S. typhimurium*, which are members of a family of two-component response regulators (156a). It has been suggested that FrzE is a second messenger that relays information between FrzCD and the gliding motor in response to aggregation and/or feeding signals. FrzG is homologous with CheB from *E. coli*, which encodes the methylesterase (156). These results suggest that *M. xanthus* controls gliding motility with a series of proteins that is evolutionarily related to those controlling flagellar rotation of the enteric bacteria.

Cooperative Growth

Myxobacteria obtain their carbon, nitrogen, and energy by degrading macromolecular organic material including other cells. To this end they secrete a variety of enzymes that hydrolyze peptidoglycans, lipids, nucleic acids, polysaccharides, and proteins (for a review, see reference 20). The

spectrum of susceptible organisms is quite broad. In general, gram-positive bacteria are more sensitive to the partially purified bacteriolytic enzyme fraction than are gram-negative bacteria, perhaps because the outer membrane of the latter shields the peptidoglycan. Prior treatment of gram-negative bacteria with agents that damage the outer membrane sensitizes them to the bacteriolytic enzymes (8, 71, 82, 175, 176, 186, 238). In some cases, direct contact between predator and prey is necessary to achieve lysis (21, 119, 149, 214, 225). Fatty acids isolated from *M. xanthus* and added to resistant eubacteria sensitized the eubacteria to lysis by bacteriolytic enzymes (82). The location of fatty acids on the myxobacterial cell surface and in the extracellular matrix (73, 74) might enhance lysis of the prey.

Growth of *M. xanthus* on insoluble proteins, such as casein, is cell density dependent. Under conditions when cells must hydrolyze protein as the sole source of carbon, nitrogen, and energy, growth in liquid requires a minimal cell density of about 10^3 cells per ml and the rate of growth increases with the cell density (216). In contrast, the growth rate in a liquid medium containing hydrolyzed casein (Casitone) does not vary with the cell density. Cooperative growth appears to be due to the cooperative hydrolysis of casein. The concentration of extracellular proteases and the amount of casein hydrolyzed are directly proportional to the cell density. These results suggest that more efficient feeding occurs at higher cell densities by communal use of the extracellular enzymes.

The growth rate on casein at low cell densities is much higher than expected given the small amount of casein hydrolyzed (216). These results could be explained if the extracellular enzymes were localized near the cell surface, where they might increase the local concentration of free amino acids. It is likely that the extracellular enzymes are sequestered in such a manner. The extracellular matrix of *Myxococcus virescens* is composed of protein, polysaccharide, and lipid (14:8:16) (73). The complex appears to serve as a passive means of acquiring nutrients by binding, denaturing, and hydrolyzing proteins. At least three extracellular proteases are observed in this complex, as well as bacteriolytic enzyme activity (74).

Myxobacteria form multicellular clumps which further increase the local concentrations of the extracellular hydrolytic enzymes. Growth of *M. xanthus* cells on the cyanobacterium *Phormidium luridum* results in large interspecies clumps in which the cyanobacterial cells are lysed (21). When *M. xanthus* is incubated in the light with *P. luridum* as the sole source of nutrients, both species persist indefinitely with 9-day predator-prey population cycling (20). In natural environments bacteria often accumulate in biofilms containing cells of many species (30). There may be a selective advantage for myxobacteria to form such multicellular associations because of the higher growth rates. Selection for factors that accentuate cooperative growth such as cell cohesion and social motility may also occur. In fact, cooperative growth may have provided the selective advantage for the evolution of many aspects of myxobacterial social behavior.

Rippling, a Periodic Behavior

Rhythmic behavior is common in eucaryotic organisms and may be divided into two basic types (for reviews, see references 54 and 79). Circadian rhythms have a period length close to, but not exactly equal to, the 24-h period of the Earth's rotation. They are entrained to the 24-h Earth cycle by external light-dark cycles whose period lengths are close to 24 h. They are also temperature compensated in that the period length is the same at different but constant temperatures. Ultradian biological rhythms occur with a higher frequency than circadian rhythms, but seem to be subject to similar control mechanisms. Biological rhythms can regulate behavioral parameters such as emergence of *Drosophila* pupae (190) or biochemical parameters such as rhythmic production of metabolic intermediates in *Neurospora crassa* (279). The period of the rhythms is regulated by an endogenous clock that has proven difficult to identify.

During time-lapse photographic studies of myxobacterial colonies, Reichenbach discovered a form of multicellular movement called rippling (because of the resemblance of this behavior to ripples on the surface of water) (197, 198). Rippling cells form a series of equidistant ridges that are parallel to each other and move in a pulsating manner so that a wave is propagated outward from the center of the colony. The entire colony produces 5-h cycles of vigorous rippling followed by a quiescent state. Myxobacteria appear to be the only procaryotes that perform this rhythmic behavior (199).

The propagation of ripples has been examined under several different conditions (234). Ripples have a wavelength of 45 to 70 μm, a period of 19 to 21 min, and a maximum velocity of 2.2 to 3.7 μm/min, which is about equal to the rate of gliding motility. The cells within a ripple are arranged at a 40° angle to the direction of wave movement, and since cells glide in the direction of their long axis, it is likely that the cells move at a 40° angle to the direction of ripple propagation. Despite the vigorous rippling activity within a colony, there is little net displacement of cells because the movement of ripples in one direction is countered by movement of other ripples in the opposite direction. The ridges of cells are organized into a series of parallel tracks 100 to 200 μm wide that move synchronously, in phase, and in the same direction (Fig. 7). Ripples in adjacent tracks move in opposite directions. At the intersection of these opposing tracks, cross-over zones occur in which the ridges split laterally along the center of the ridge line into two parts that connect with ridges in adjacent tracks. Because of the 40° angle of cell movement, cells would eventually cross from one track into another and, in doing so, be forced to change their direction of movement. Time-lapse studies of individual cells should reveal the precise cyclic trajectory.

Rippling is observed in situations in which there is substantial hydrolysis of eubacterial cell walls, for example when myxobacteria prey on *Micrococcus luteus* cells (198, 234). Rippling is induced by peptidoglycan and its components *N*-acetylglucosamine, *N*-acetylmuramic acid, diaminopimelate, and D-alanine (234). Myxobacteria secrete a variety of enzymes capable of hydrolyzing peptidoglycan into small, soluble fragments (81, 254). The presence of rippling cells appears to be a sensitive indicator of the presence of extracellular peptidoglycan components.

Rippling is dependent on both A and S motility systems (234). Since some of these motility mutants, particularly the A-system mutants, form fruiting bodies (88), it is likely that rippling is not required for fruiting-body morphogenesis but is incidental when it is induced by the release of peptidoglycan. The most intriguing gene requirement for rippling is the *csgA* gene (234), which is also essential for development. CsgA is an extracellular polypeptide that may be a developmental morphogen (77, 115, 116, 231, 233, 236). Since it is possible to isolate *csgA* mutations that disrupt sporulation but not rippling (231), it is likely that CsgA contains different domains for each behavior.

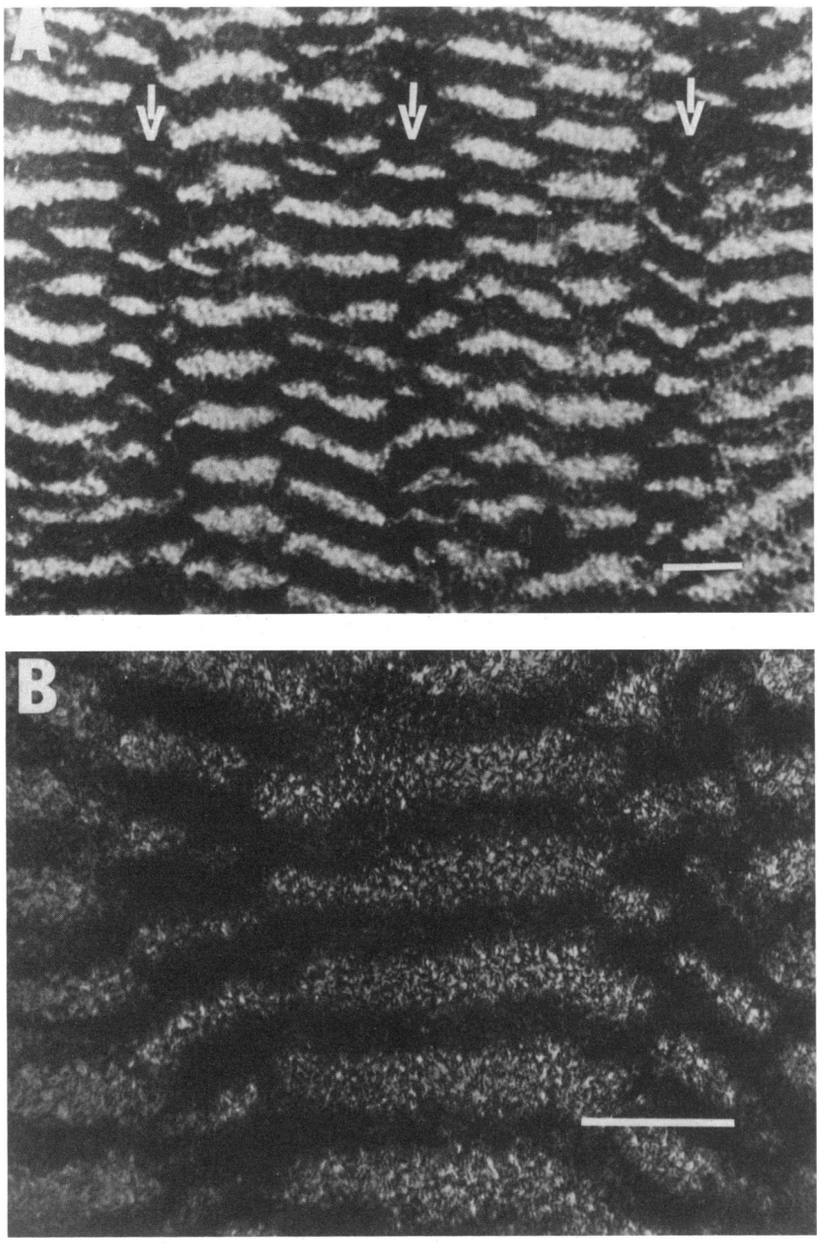


FIG. 7. Organization of *M. xanthus* ripples into tracks (234). (A) Ripples in adjacent tracks move in opposite directions and are separated crossover zones, some of which are indicated by arrows. (B) One track of ripples flanked by two crossover zones. Individual cells are visible in the troughs between ripples. Bars, 100 μm. (Reprinted with permission.)

Rippling and fruiting-body formation appear to have little in common except for the need for motility and the CsgA protein to establish morphogenetic patterns. The mechanism by which the wave is propagated is under investigation. One possibility is that pulses of CsgA are emitted in a manner analogous to cyclic AMP secretion during aggregation of *Dictyostelium* cells (256). It has been observed that motility is essential for CsgA signaling during development (117, 122). If perception of CsgA caused cells to move and cell movement induced CsgA secretion, a positive feedback loop would be created, resulting in waves of ripples (122).

Fruiting-Body Morphogenesis

The mechanism by which the genetic code specifies the formation of a three-dimensional multicellular pattern is one of the foremost problems in developmental biology. Since myxobacterial development is induced by nutritional stress, the contribution of cell proliferation to the development of the multicellular pattern appears to be minimal. Rather, the fruiting body forms from the ordered migration of cells. Fruiting-body morphogenesis can be divided into several stages. First, there is a switch from the foraging behavior observed during vegetative growth to the aggregation behav-

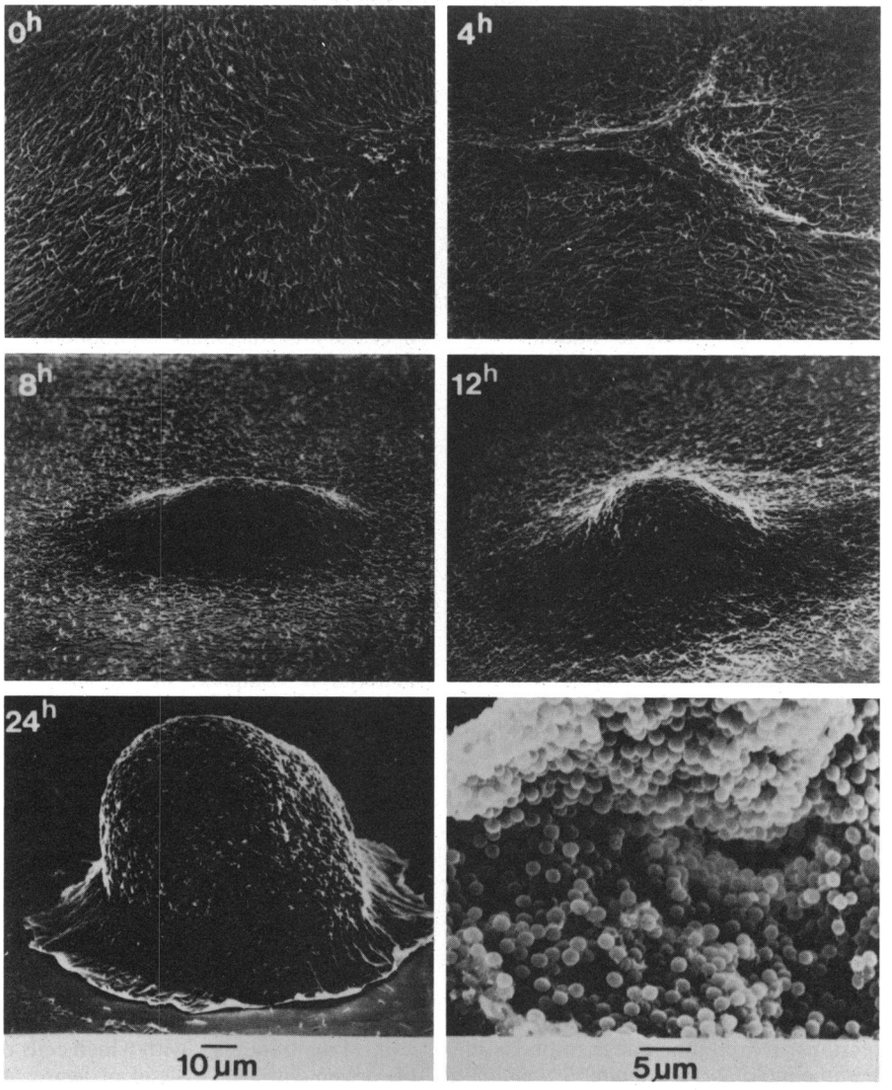


FIG. 8. Scanning electron micrographs of *M. xanthus* cells undergoing development (127). (Reprinted with permission.)

ior observed during development. Second, the directed movement of cells results in the formation of a large aggregate. Third, the fruiting body is constructed from the cell aggregate. This last step varies in complexity with the different myxobacterial species depending on the size and shape of the fruiting body. The formation of simple fruiting bodies by *M. xanthus* will be compared with the formation of complex fruiting bodies by *S. aurantiaca* in the following sections.

M. xanthus. Fruiting-body morphogenesis of *M. xanthus* has been studied by scanning electron microscopy (127, 181, 237) and time-lapse phase microscopy (204, 206). In one study cells were observed to be arranged in a small spiral at the initiation of development (181). Cells accumulate at the aggregation focus in stacked layers that have a terraced appearance (127) (Fig. 8). As aggregation continues, a hemispherical mound of cells forms which enlarges throughout the aggregation process. Sporulation begins prior to the completion of the mound; it starts in the interior region of the mound and seems to progress toward the periphery (181).

Little is known of the sequence of events leading to the initiation of aggregation. It appears that at least one diffusible molecule is involved since *Myxococcus* fruiting bodies on one side of a permeable barrier induce vegetative cells on the other side of the barrier to form fruiting bodies and both sets of fruiting bodies are in alignment (136, 162). The most likely candidate is an adenine derivative, because certain adenine-containing compounds induce cells to develop at nonpermissively low cell densities (232) or nonpermissively high nutrient levels (22, 148). Evidence that secreted purines are required for fruiting-body morphogenesis comes from either inhibiting purine de novo synthesis or by binding extracellular purines with Norit (232). Hadacidin, a competitive inhibitor of adenylosuccinate synthase, inhibits fruiting-body formation, and myxospores form in an even layer over the agar surface. The inhibition is reversed by addition of exogenous adenosine. When Norit is incorporated into the agar, sporulation also occurs uniformly over the agar surface. The most abundant extracellular purine, adenosine, which is produced at a local concentration of 6.7 μM, induces aggregation of cells at nonpermissively low cell densities, suggesting that adenosine or a metabolite of it plays an essential function. Two functions for adenosine have been suggested. First, the extracellular concentration

of adenosine could provide the cells with a means of assessing nutritional stress and/or an adequate cell density for development (232). Adenosine salvage might deplete the cellular concentration of 5-phosphoribosyl-1-pyrophosphate, a substrate for the synthesis of several amino acids, purines, and pyrimidines, thereby subjecting the cells to more extreme starvation and initiating development (148). Second, adenosine could stimulate the aggregation process, perhaps by serving as a biochemical substrate for the synthesis of a compound involved in aggregation (229). It is unlikely that morphogenetic gradients of adenosine are necessary for development, since extremely high adenosine concentrations do not inhibit fruiting-body formation.

Both of the multigene motility systems appear to be necessary for aggregation. About 10% of the A^-S^+ mutants and 67% of the A^+S^- mutants fail to form fruiting bodies (88). How the A and S motility systems are modified to shift from foraging behavior during vegetative growth to developmental aggregation remains unknown. One possibility is that methylation of LPS is used to limit adventurous behavior during aggregation. During aggregation, methylation of LPS is observed (188, 189). The methylated carbohydrate is 6-*O*-methylgalactosamine and seems to be found only on extremely high-molecular-weight LPS molecules, possibly in the most exterior portion of the O antigen (189). Certain O-antigen mutants which lack adventurous motility aggregate normally, indicating that the O-antigen carbohydrate(s) affected by these mutations is not essential for aggregation (57–59). A careful biochemical characterization of the LPS from the mutant strains should reveal whether the mutations affect the methylated galactosamine residue.

On examining the films and photographs of aggregating cells it becomes clear that the directed movement of cells into the fruiting body is different from the chemotactic movement of enteric bacteria or the cellular slime molds. Chemotaxis of enteric bacteria is a biased random walk in which the direction of cell movement changes many times during chemotaxis (160). Net movement up the gradient occurs because cells swim up the gradient for longer intervals than in other directions. Developmental chemotaxis of the eucaryotic cellular slime mold *Dictyostelium discoideum* occurs in a rhythmic manner that corresponds to release of pulses of the chemoattractant, 3′,5′ adenosine monophosphate (256). With *M. xanthus*, streams of cells moving directly into the aggregation center are observed, and random directional movement or rhythmic cell behavior is not observed (127, 181, 204, 206, 237).

Genetic evidence suggests that there are at least two systems of directed movement that lead to aggregation. Only one of these aggregation systems has been identified, and it is referred to as *tag* (temperature-dependent aggregation) (179, 182, 257). The aggregation systems differ in their temperature sensitivity. All *tag* mutants, even those formed by transposon insertion, are able to aggregate at 28°C but not at 34°C, in contrast to wild-type cells, which can aggregate at both temperatures. These results suggest that the *tag* system is the only aggregation system that operates at 34°C. The *tag* locus is 8.5 kilobase pairs (kbp) long and contains nine genotypic complementation groups (182).

The mechanisms of directed movement probably do not involve simple chemotaxis. *M. xanthus* is not attracted to a variety of diffusible substances including amino acids, cell wall components, nucleotides, and cell extracts (48). One possibility, based on elasticotaxis (243), is that the polysaccharide chains in the extracellular matrix on which the cells move assume an orientation that favors cell movement in one direction only (260). Another possibility is that molecules associated with the extracellular matrix direct cell movement.

Evidence in support of a model involving the extracellular matrix comes from mutant studies. Pairwise mixture of certain nonaggregating mutants stimulates one or both to form fruiting bodies (76, 161, 228). One of the most carefully documented studies involved a group of social motility mutants known as Dsp mutants. Mixture of *dsp* cells with *igl* cells, or *dsp* cells with wild-type cells resulted in substantial aggregation of the *dsp* cells, suggesting that the *igl* cells and the wild-type cells were able to supply *dsp* cells with the necessary extracellular components for aggregation (228). Stimulation did not occur when the strains were separated by a dialysis membrane, indicating that the necessary factor is not a small molecule. The *dsp* mutants cannot produce the extracellular matrix material and as a result are noncohesive (3, 228). The matrix material is produced by both *igl* and wild-type cells, which are cohesive. Mixing *dsp* cells with either *igl* cells or wild-type cells results in the formation of macroscopic cell clumps containing cells of both types (228). It appears, then, that social motility, extracellular matrix formation, and cell cohesion are essential for fruiting-body morphogenesis.

The chemical composition of the extracellular matrix material is complex. In *M. virescens* it is composed of polysaccharide, protein, and lipid (73). The principal monosaccharides contained in polysaccharide secreted during vegetative growth of *M. xanthus* are mannose, D-glucose, D-galactose, and hexosamine, and these are also present at approximately the same ratio in developmental exopolysaccharide (251). Apparently, there is considerable use of preexisting enzyme systems in synthesizing the developmental polysaccharides. Structural analysis of these carbohydrates is likely to be an important contribution that is necessary for determining the mechanism of this and other social behaviors.

Another promising approach to understanding the mechanism of directed movement involves the *frz* genes, which control the frequency with which cells reverse their direction of movement (11). Instead of aggregating, *frz* mutant cells sporulate in long thin streams (269, 283). The *frz* genes are homologous with chemotaxis genes of enteric bacteria (see the section entitled "Other loci controlling gliding-cell behavior"). FrzCD contains a region of about 250 amino acids that is similar to the methyl-accepting chemotaxis receptor protein, Tar, of enteric bacteria (154) and is modified by methylation (156). If the methylation state of FrzCD is influenced by environmental or intracellular signals that control fruiting, analysis of the methylation of this protein may provide an assay for the identification of such signals. Expression of the *frz* genes increases as much as 10-fold during aggregation (269). The *tagE* gene may regulate *frzCD*, since a *tagE* mutation caused increased and prolonged expression of *frzCD* (269).

Because social motility is involved in fruiting-body morphogenesis, developmentally regulated changes in the cell surface probably play an important role. The outer membrane of *M. xanthus* is similar to that of the enteric bacteria in that it is composed of LPS, protein, and phospholipid (184). The cell surface undergoes a remarkable number of transformations during development. The relative abundance of various cell surface antigens was examined through the use of monoclonal antibodies raised against preparations of developing cells (65, 66). More than three-quarters of the hybridoma lines reacted with antigens that were observed on

both vegetative and developing cells. Of these antigens, 57% decreased in concentration, some disappearing completely, while 9% increased in concentration. One of the most abundant cell surface proteins is a glycoprotein known as VGP, which accounts for 1% of the total cell protein during vegetative growth, but virtually disappears during development (143, 144). The remaining one-quarter of the hybridoma lines reacted with cell surface antigens that were not detected in vegetative cells (65, 66).

MAb 1604 is directed against a protein antigen with a molecular mass of about 51 kilodaltons (kDa) (104). There are about 2,400 molecules of the antigen per cell, and the antigen appears to be distributed in about 30 clusters along the cell periphery. MAb 1604 fab fragments inhibit aggregation, fruiting-body formation, and sporulation (67, 105). Tn*5* insertion was used to isolate a mutation in the 1604 antigen (57). The mutant contained less than 4% of the antigen molecules observed on the wild type, but maintained its ability to develop. There are several possible reasons for these conflicting results. The insertional mutant may produce a truncated protein that lacks the MAb 1604 epitope but maintains the portion of the protein that is necessary for biological function. Alternatively, treatment of cells with MAb 1604 may mask other local antigens that are essential for development. The 51-kDa protein that is the target of MAb 1604 forms a complex with a 23-kDa protein (105). MAb 4054, which binds to the 23-kDa protein, also disrupts development.

Several new membrane proteins appear during the early stages of development (185). The most prominent membrane-associated protein is an erythrocyte lectin known as myxobacterial hemagglutinin (MBHA), which makes up 1 to 2% of the total cell protein (31). It has a molecular weight of 27,920 as deduced from the nucleic acid sequence of the gene (212), 27,000 by gel filtration, and 30,000 by velocity sedimentation on sucrose gradients (32). In general, lectin activity is due to multimeric associations that form bridges between the two cross-linked cells. Since MBHA behaves as a monomer in solution, its lectin activity is likely to be due to an internal repeated structure that allows it to interact with receptors on two different cells. It does contain four highly conserved domains of 67 amino acids, and it is possible that these interact with the MBHA receptors (212). MBHA binds to complex carbohydrates that contain a β-linked galactose residue (31). Both vegetative and developing cells contain receptors for MBHA, but the receptors are different. Binding of MBHA to vegetative cells is inhibited by the glycoprotein fetuin, whereas binding to developmental cells is not (33). Furthermore, developing cells have a higher association constant for MBHA than vegetative cells do. MBHA appears to be localized at the cell poles in developing cells (169). Strains containing a null mutation in the *mbhA* gene are delayed in their development in the absence of exogenous Mg^{2+} (213).

S. aurantiaca. At least three episodes of directed movement are involved in fruiting-body formation of *S. aurantiaca*; they are depicted in the scanning electron micrographs shown in Fig. 9. One of the earliest signs of an aggregation focus is a layer of cells that is encircled by a ring of cells (260). It appears that the cells move in a spirallike trajectory through the ring and into the aggregate. This ring persists throughout the aggregation process (75, 260). When the aggregate reaches a size comparable to that of the *M. xanthus* fruiting body, the cells at the base of the aggregate become oriented vertical to the agar surface; this initiates the second episode of directed movement, which leads to the formation of a stalk (260). The stalk continues to elongate until it reaches a length of more than 100 μm. At the top of the stalk the third type of directed movement is initiated as cells begin to migrate away from the aggregation focus in several different directions. Budlike swellings appear which elongate to become sporangioles that remain attached to the stalk by pedicels. Inside the sporangioles the thousands of vegetative cells differentiate into myxospores (262). The stalk is composed of cells that are aligned parallel to the long axis of the stalk and eventually die (75, 262).

The most striking difference in the regulation of *S. aurantiaca* fruiting-body formation and that of *M. xanthus* is the requirement for light (192, 193, 247). In the absence of light, *S. aurantiaca* cells at low cell densities aggregate into interconnecting ridges that never mature into fruiting bodies and fail to differentiate sporangioles and myxospores (192, 193). Blue light in the range of 400 to 500 nm appears to be the primary stimulus, although a high irradiance of far-red light also stimulates morphogenesis (272). Light induces the expression of at least one protein and enhances the expression of several others (101). Recent studies on the photoinduction of carotenoid biosynthesis in *M. xanthus* have revealed the genetic system by which blue light regulates transcription (7, 150, 151).

A pheromone is secreted during the aggregation and early stalk formation of *S. aurantiaca* (247). It has been partially purified and has a molecular weight of less than 1,800. It is soluble in chloroform and methanol, but is resistant to phospholipase and acid phosphatase. It appears to be active at concentrations as low as 10^{-13} M and does not stimulate development of *M. xanthus*. High concentrations of the pheromone can substitute for light, suggesting either that light sensitizes cells to the action of the pheromone (247) or that the requirement for light precedes or induces pheromone synthesis. The role of blue light and pheromone in the regulation of *S. aurantiaca* morphogenesis remains a promising area for future investigation.

Development of *S. aurantiaca* is stimulated by guanine derivatives. Guanosine at a concentration of about 3 μM can substitute for either light or pheromone (247). A combination of light, pheromone, and guanine derivative is more effective in stimulating development than any of the treatments alone. It is not known whether a guanine-containing compound is secreted during development.

Sporulation

The myxospore is an asexual, dormant cell that can survive hostile environmental conditions for many years. Sporulation of *M. xanthus* is an encystment in which the rod shortens and assumes a spherical shape. Remodeling of the peptidoglycan layer occurs; the amount of peptidoglycan relative to the surface area of the cell increases along with the amount of cross-linking between the peptide side chains (108, 271). There is an increase in the autolytic potential of the cell which presumably reflects an increase in the activity of peptidoglycan hydrolytic enzymes (35, 120). Novel spore envelope polysaccharides are synthesized (5, 121, 251), and one of the major spore storage products is the disaccharide trehalose (155). Accordingly, substantial metabolic energy is extended in the direction of gluconeogenesis (55, 56, 183).

The ultrastructure of fruiting-body spores differs from that of glycerol-induced spores particularly with regard to the spore coat (Fig. 10). Glycerol spores have a thin (20-nm) spore coat, whereas fruiting-body spores contain a thick coat composed of several layers. The major component of the

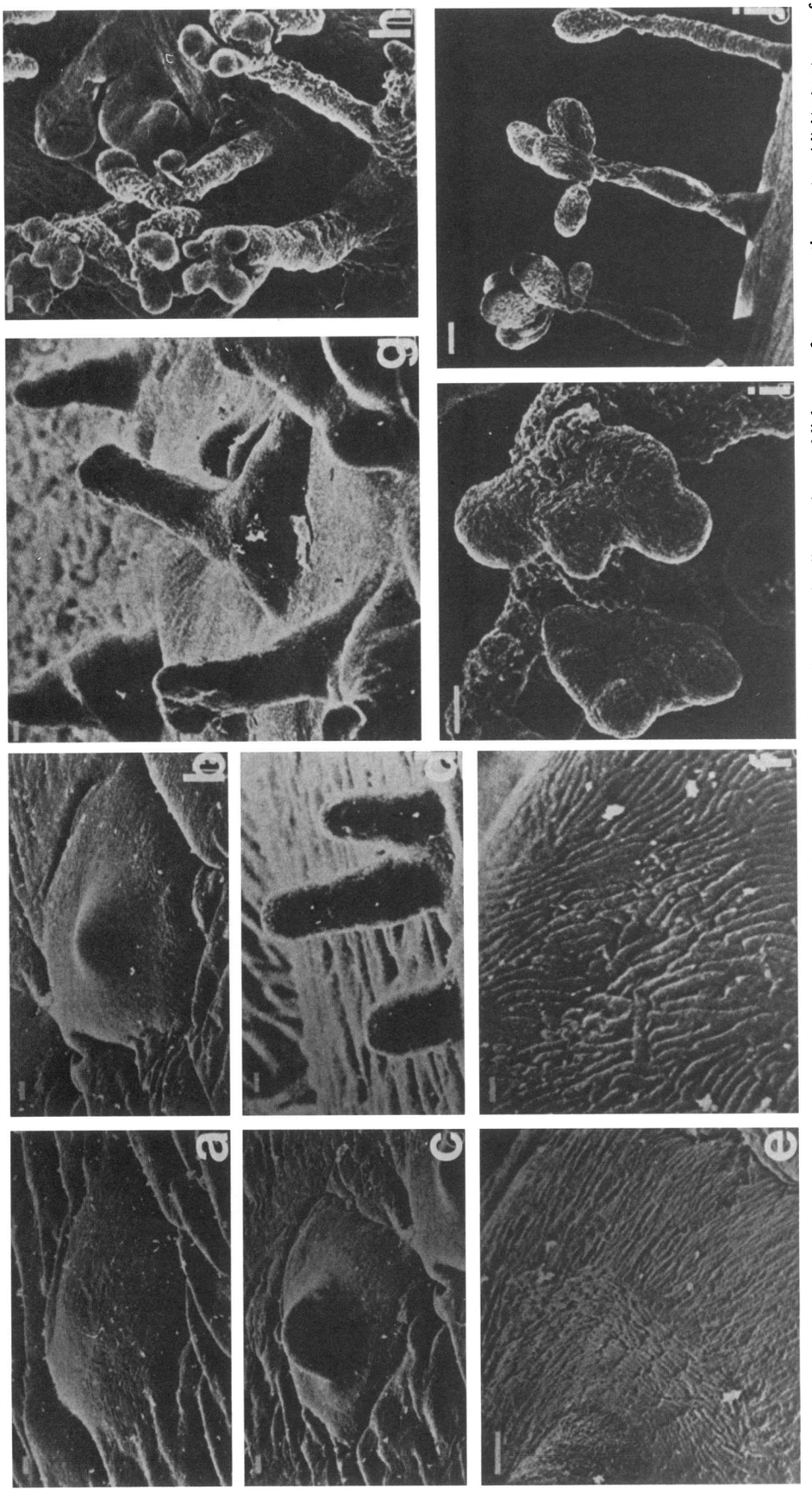


FIG. 9. Scanning electron micrographs of *S. aurantiaca* cells undergoing development (75). (a to c) Events leading to the establishment of an aggregation center. (d) Early stages of stalk formation. (e) Higher magnification of ring of cells encircling aggregate seen in panel c. (f) Higher magnification of the tightly packed cells seen in panels c and e. (g) Completed stalk. (h and i) Initiation of sporangiole formation at the apex of the stalk. (j) Mature fruiting bodies. Bars are 1 μm in panel f, 5 μm in panel e, 10 μm in panels a to d and i, and 20 μm in panels g, h, and j. (Reprinted with permission.)

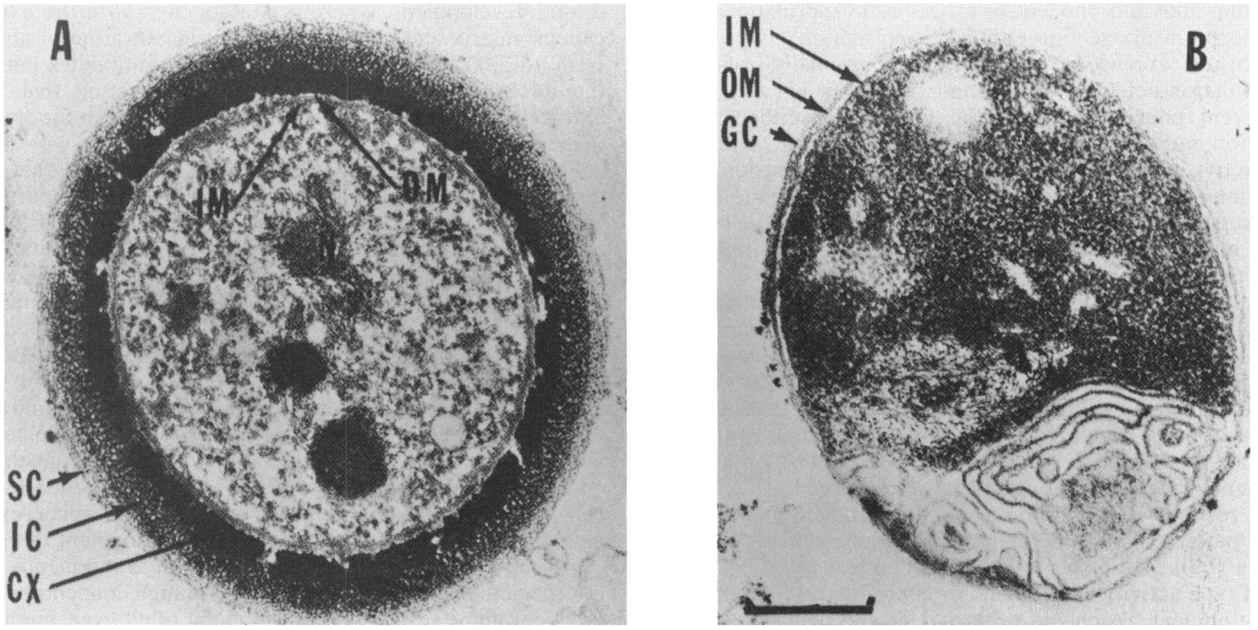


FIG. 10. Transmission electron micrographs of thin sections from myxospores of *M. xanthus* (93). (A) Fruiting-body myxospore. (B) Glycerol myxospore. Bar, 0.5 μm. Abbreviations: N, nucleoid; IM, inner membrane; OM, outer membrane; CX, cortex; IC, intermediate coat; SC, surface coat; GC, glycerol spore coat. Micrograph was prepared by Robert Silverstein. (Reprinted with permission.)

outermost layer of fruiting-body spores is protein S, which can be extracted from spores with 1 M NaCl or (ethylenedinitrilo)tetraacetate, in which case the outer 30-nm layer of the spore coat disappears (93). Purified protein S spontaneously assembles on the spore surface in the presence of Ca^{2+} and saturates at about 3.3×10^6 molecules per spore, a level 1.35 times higher than the normal level. The two high-affinity calcium-binding domains of protein S are similar in amino acid sequence to those of calmodulin and appear to be necessary for assembly of protein S on the spore surface (95, 100, 253). Each of the Ca^{2+}-binding domains contains a serine residue, and replacement of both of the serine residues with arginine residues prevents calcium binding and self-assembly of protein S on the spore (253).

The protein S gene, *tps*, has extensive identity with an adjacent gene known as *ops* (99, 254). Although both genes are developmentally regulated at the transcriptional level, they are expressed at different developmental stages (42, 44, 252, 254). Expression of *tps* is induced by starvation and begins about 5 h into development. Protein S is the most abundant developmental protein, reaching a level of about 15% of the total protein at the peak of its synthesis (93). This appears to be due, at least in part, to the fact that the *tps* mRNA has a long half life (38 min) (171). In contrast, *ops* expression begins at the onset of sporulation and its gene product accumulates in much smaller quantities inside the spores (41, 42, 44, 254).

The developmental regulation of *ops* and *tps* has been more intensively studied than that of any other developmental genes. The regulatory regions of *ops* and *tps* are unusually large and seem to contain both positive and negative control elements (43). A transcriptional enhancerlike element that is located just upstream of the *ops* promoter is involved in the developmentally regulated expression of *ops* (113). This enhancer is also essential for the developmental expression of *tps* nearly 2 kbp downstream. The enhancer element is active in either orientation and at distances up to 15 kbp away from the transcriptional start site for *tps*. Several specific DNA-protein complexes are observed after incubation of the DNA segment containing the enhancer with an extract of early developmental cells. Extracts of vegetative cells contain much less of the enhancer-specific DNA-binding activity. These results are consistent with a model in which a positive regulatory protein binds to the enhancer and activates transcription of *tps* (113).

The differences in the architecture of fruiting-body and glycerol spores may occur because glycerol sporulation bypasses many of the early developmental steps including induction of protein S synthesis. The *tps* gene is not expressed during glycerol-induced sporulation (41, 42), providing one reason for the absence of this protein on the surface of glycerol spores. Glycerol spores also lack the protein S receptor and cannot assemble purified protein S on their surface (93, 96).

Cell-cell interactions during sporulation. Nearly 30 years ago it was discovered that pairwise mixtures of certain developmental mutants resulted in normal sporulation (161). The germinated spores from such mixtures had the original mutant phenotypes, indicating that extracellular complementation did not involve a permanent genetic change. The authors postulated that the mutants were defective in producing an essential developmental compound but could respond to that compound when it was produced by mutants of a different type. These findings were later extended by a larger mutant search (76). About half of the known nonsporulating mutants are conditional mutants that may be stimulated to sporulate by contact with wild-type cells or other nonsporulating mutants. Pairwise mixtures of different mutants allowed definition of four extracellular complementation groups (76). The four mutant groups have been designated Asg, Bsg, Csg, and Dsg (for A signal, etc.). These mutants have been intensively investigated with the hope of identifying intercellular signals that guide development. It is important to note that these mutant groups are defective in

fruiting-body morphogenesis as well as in sporulation. Their inclusion in the section entitled "Sporulation" is primarily for historical reasons, since they were originally isolated as sporulation-defective strains and since they have distinctly different phenotypes from the mutants described in the section entitled "Fruiting-body morphogenesis," which are defective in aggregation but not sporulation. Inclusion in this section is not meant to imply that the genes function solely in sporulation. In fact, they appear to act early in development and may play a role in both sporulation and fruiting-body morphogenesis.

All of the *asg* mutations map to one of three genetic loci, *asgA*, *asgB*, or *asgC* (129, 153). In extracellular complementation assays with wild-type donor cells, *asg* mutants exert a dominant negative effect on the wild-type sporulation. In such mixtures, sporulation of the wild-type cells is suppressed by as much as 70-fold while sporulation of the *asg* mutant is stimulated as much as 1,100-fold. Developing asg^+ cells produce a heat-labile substance, referred to as A factor, that restores both morphological development and the normal pattern of developmental gene expression to *asg* mutants (130). The *asg* mutants produce less than 5% of the wild-type activity (129). A factor is produced at the onset of development, reaches a peak at 2 h, and declines to near-vegetative levels by 9 h (130). The addition of certain amino acids restores development to *asg* mutants (129). Amino acid starvation is the major environmental stimulus for the initiation of development (45), and gradual starvation is more likely to lead to rapid and synchronous development than is sudden starvation (146, 147). However, specific amino acids or peptides could stimulate transcription of developmentally regulated genes and play a regulatory role in development as well (130).

Several of the *bsg* mutations map to a gene known as *bsgA* (70). The *bsgA* mutants aggregate very little on their own and substantially delay the development of wild-type cells when present as a mixture (69). Within such mixtures, the wild-type cells seem to develop faster than the mutant cells. One possibility is that the *bsgA* mutants overproduce an inhibitor of development that is destroyed by the B signal. Despite the asynchrony, extracellular complementation between *bsgA* and $bsgA^+$ cells produces approximately equal ratios of the two spore types and restores the normal pattern of developmentally regulated gene expression (69). MAb against BsgA was used to examine the size and cellular location of the BsgA gene product (68). The antibody reacts with an 89-kDa protein, which is not produced by *bsgA* cells. The protein is produced in vegetative cells, and its concentration remains relatively constant through the first 12 h of development. More than 90% of the protein is located in the cytoplasm, and the remainder is associated with the inner membrane. The mechanism of extracellular complementation with this group is particularly puzzling. Since the BsgA gene product is not extracellular during development, it may function in the synthesis or secretion of an extracellular molecule.

All Csg mutants contain a mutation in the same gene, *csgA*, which encodes a protein with an apparent molecular mass of 17.7 kDa (77, 231, 233). Vegetative cells express this gene weakly, and expression increases about threefold during development (77). Since CsgA is a minor component of cells, it was overproduced in *E. coli* to obtain enough protein to examine its developmental function in more detail (236). Immunopurified polyclonal antibodies raised against the CsgA protein were used to determine the cellular location of the protein in developing *M. xanthus* cells by colloidal-gold labeling and electron microscopy. CsgA is extracellular during development, where it is associated with the extracellular matrix and cell surface at a concentration of about 1,000 to 2,000 molecules/cell. Anti-CsgA antibodies inhibit the development of wild-type cells, suggesting that the extracellular presence of this molecule is essential for development.

Several laboratories have identified compounds that induce *csgA* mutants to sporulate. DS, a partially purified factor of unknown composition that was isolated from $csgA^+$ cells, rescues the development of *csgA* mutants that have an *sglA* mutation, but not of *csgA* mutants in an otherwise wild-type background (103). Since *csgA* mutants in both the $sglA^+$ and *sglA* background are stimulated to sporulate in the extracellular complementation assay, DS is probably not the actual C signal. Several carbohydrates and amino acids that are biochemical precursors to peptidoglycan also induce *csgA* mutants to sporulate. *N*-Acetylglucosamine, *N*-acetylmuramic acid, D-alanine, diaminopimelate, and certain combinations of these chemicals induce *csgA* cells to form wild-type levels of spores (235). However, even high concentrations do not rescue the development of *sglA csgA* cells, suggesting that peptidoglycan components are not the actual C signal (103). Relatively high concentrations of glucosamine rescue the development of all *csgA* mutants tested, but the concentration is too high for glucosamine to be the C signal (103). These results suggest that many compounds that are not the actual signals are capable of restoring development by reinitiating the developmental pathway at some point downstream from the mutational block. More recently, a 17-kDa protein known as C factor was extracted from wild-type cells and found to restore development of *csgA* mutants (115, 116). C factor, which is undetectable in *csgA* mutants, is active at a concentration of 2 nM and appears to be the product of the *csgA* gene. Anti-CsgA antibodies cross-reacted with purified C factor (116, 236). Furthermore, the N-terminal amino acid sequence of an internal peptide of C factor is identical to that predicted from the DNA sequence of the *csgA* gene (115). Taken together, these data make a strong argument that the CsgA gene product is the C signal.

Nonmotile mutants abort the developmental pathway at about the same place as do *csgA* mutants (122). Mgl^- mutants are unable to serve as CsgA donors or recipients in the extracellular complementation assay in spite of the fact that they produce a biologically active CsgA protein (117). These results suggest that motility is essential for CsgA transmission. One possible role for motility could be achievement of an optimal cell alignment for signal exchange (117a).

CsgA mutants are unable to ripple or to form fruiting bodies, both processes that require directed cell movement (234). The two most likely roles for CsgA are a functional role in directed movement, possibly as an attractant, or a regulatory role in inducing genes controlling directed movement. Pseudoreversion genetics was used to distinguish between these possibilities (209). If CsgA is the sole attractant, it should not be possible to isolate second-site suppressor mutations that restore rippling and fruiting body formation. On the other hand, if CsgA induces expression of genes responsible for directed cell movement, it should be possible to isolate mutations in other components of the regulatory network that restore directed movement. By taking advantage of the fact that spores are much more resistant to heat and sonic stress than vegetative cells (76, 248), sporulating pseudorevertants were selected from *csgA* mutants (209). The suppressor mutations mapped to six different loci and

exhibited a variety of phenotypes. One group, which contains two alleles that were formed by transposon insertion, exhibited normal rippling and fruiting body formation in spite of the absence of CsgA. These results suggest that CsgA plays a regulatory role rather than a functional one. The suppressor mutations will undoubtedly be helpful in revealing the structure of the regulatory network.

Two of the *dsg* mutations map to a gene known as *dsgA* (26, 27, 135). Although some point mutations in the gene affect only development, transposon insertions in the gene are lethal, suggesting that *dsgA* has an essential vegetative function (27). Treatment of *dsgA* mutants with the autocide AMI restores fruiting-body formation and sporulation (219). AMI is produced from the phospholipase-mediated hydrolysis of the major membrane phospholipid phosphatidylethanolamine (63). AMI is a mixture of saturated and unsaturated fatty acids that accumulate in the culture medium during vegetative growth and kill the producing cells (258, 259). All AMI-resistant mutants are unable to form fruiting bodies, but these mutants have not yet been subjected to genetic analysis to determine whether a single mutation causes both AMI resistance and the inability to develop (63). Whether AMI is the actual D signal is unclear. In lysing a small population of developing *dsgA* cells, AMI treatment may release the true D signal or other substances that can bypass this developmental step.

The Asg, Bsg, Csg, and Dsg mutants are defective in steps leading to the initiation of sporulation, but not in the sporulation process itself. This is shown by the fact that they form normal levels of morphologically complete and physiologically resistant spores in response to chemical induction with glycerol (26, 235). Furthermore, the intercellular signaling steps occur early in development, prior to the initiation of sporulation. Mutants defective in sporulation per se have been isolated but have not been extensively studied.

During cell enumeration studies and radiolabeling experiments, Wireman and Dworkin observed that a substantial number of cells (70 to 90%) died while developing on an agar surface (274, 275). O'Connor and Zusman adapted the [^{3}H]thymidine-labeling technique of Wireman and Dworkin to submerged culture and observed that no more than 20% of the cells die during development (180). Rosenbluh and Rosenberg observed that sporulation in liquid shake cultures was preceded by a large decrease in turbidity, although they did not investigate the reason for the turbidity decrease (220). To determine the extent of cell death during development, Rosenbluh et al. embedded small numbers of cells in 25-μm-diameter agarose beads and incubated the beads in a nutrient-free buffer at a high bead density (218). They observed that sporulation was preceded by a decrease in the average number of cells per bead. As many as 82% of the cells lysed, while 44% of the surviving cells sporulated. Since all four studies were done under different developmental conditions, it would appear that the method used to cultivate developing cells has a major influence on the extent of lysis.

Whether there is a specific developmental function for autolysis and what that function might be remain to be determined, but many possibilities have been suggested. First, catabolism of lysed cells may serve as an energy source for development under some circumstances. This notion fits with the predatory nature of myxobacteria and their ability to scavenge protein from dead cells. Second, autolysis may provide a specific intercellular stimulus for development. AMI-induced lysis restores development to *dsgA* mutants (219), but whether this is the natural role for AMI remains to be determined. Third, lysis products may serve as a stimulus or substrate for the initiation of sporulation (273, 275). An experiment in support of this notion showed that sporulation was induced only when there were high concentrations of lysis products or other nutrient sources. Fourth, lysis may serve to release protein S where it spontaneously assembles on the spore surface (252). Protein S does not contain a hydrolyzed leader peptide, and the N-terminal region is not especially hydrophobic. However, protein S could also be transported out of the cell by nonconventional means. Localization of protein S was examined by fractionating pulse-labeled developing cells into osmotic-shock, cytoplasmic, and spore fractions (172). After accumulating in the cytoplasm for many hours, protein S appears in the periplasm about the time of sporulation. The authors suggested that protein S is transported out of the cell in stages. Finally, autolysis may occur simultaneously with development under some circumstances but may have no essential developmental function. The developmental function of autolysis, if any, remains a controversial issue.

Involvement of the phase variation. *M. xanthus* undergoes a phase variation that appears to be involved with the developmental cycle (103, 274). Two types of colonies are usually apparent on a plate, one yellow and the other tan (18, 19). Although the most obvious trait affected by the phase variation is the pigmentation type, other features of the cell are also affected. Tan variants are usually less social in their motility, are less cohesive, and produce less of the extracellular matrix material than yellow cells. The proportion of yellow and tan cells in the population is affected by environmental factors. Growth on a rich medium at low cell densities tends to increase the proportion of yellow cells. Tan variants predominate in dense populations and preferentially form myxospores during fruiting-body sporulation. This variation may have an underlying logic that is related to the social behavior of the cells. Under growth conditions that include ample nutrient to support the population, the more social yellow cells are produced because adventurous behavior is not necessary to locate additional food sources. In contrast, the more adventurous tan variants, which predominate in the myxospore population, would aggressively search for new food sources following germination. Little is known of the mechanism of this variation, and it remains a fascinating area for future work.

NUTRITIONAL CONTROL OF DEVELOPMENT

There has been little work on the nutritional regulation of development over the past 10 years. Readers interested in this topic are encouraged to consult two reviews on the subject (226, 229).

ORGANIZATION OF THE DEVELOPMENTAL PATHWAY

The developmental cycle of eucaryotic organisms has two major functions: the generation of morphological and biochemical diversity by specialization of cell types, and the generation of genetic heterogeneity through sexual reproduction. In some simpler eucaryotes, the production of asexual spores provides a third function. In procaryotic organisms development is rare. For example, not a single archaebacterium with a developmental cycle has been described. Eubacteria that have developmental cycles use them for two purposes: the generation of morphological and biochemical diversity, and the production of asexual spores.

Procaryotic developmental cycles do not emphasize sexual reproduction.

A developmental pathway mediates the transition between two cellular or multicellular states and is often regulated by DNA-binding proteins that control the expression of genes in a precise temporal order (139). Developmental gene expression is also topologically precise in that certain genes are expressed only in specific compartments or tissues (139, 224). Two general strategies, which are widely distributed taxonomically, are used in the regulation of developmental gene expression. In ancestral control, the fate of a cell is determined entirely by the lineage of the cell at each division, resulting in a mother cell plus a new cell type. The nematode *Caenorhabditis elegans* develops by such a precise pattern of cell divisions that a somatic cell at a particular position in the body has the same lineage in every organism (250). Ancestral control is also found in procaryotic organisms (173). The *Caulobacter* stalked cell divides asymmetrically into a nonmotile stalked mother cell and a motile swarmer cell. In positional control, the developmental pathway is regulated by interactions with neighboring cells or tissues. Again, this mode of control is found in both eucaryotes and eubacteria, with the most prominent procaryotic examples being the development of the myxobacteria, actinomycetes, and heterocyst-forming cyanobacteria.

Analysis of the myxobacterial developmental pathway has used both morphological and molecular markers. Morphological markers involve changes in cell position and shape with time. Molecular markers include new proteins (92, 114, 170) or enzyme activities (269) in cell extracts, new cell surface antigens (65, 66), and the expression of development-specific genes (42, 69, 123–125). The production of new markers is generally due to changes in gene expression, and the molecular markers are expressed in a defined temporal order (42, 65, 69, 92, 123–125). Identification of sigma factors (94) and DNA elements (113) that regulate developmental gene expression is in progress. The morphological and genetic aspects of the developmental pathway are obviously coupled. Mutations in essential developmental genes disrupt both the morphological manifestations of development and the appearance of molecular markers.

To determine how many developmentally regulated genes there are, the genome was mutagenized with Tn*5lac*, a transposable element and promoter probe, and the phenotype of strains containing the transposon insertions was examined (125). About 8% of the genome increases expression during development. Given a genome size of 9,454 kbp (25) and an average gene size of about 1 kbp, these results suggest that about 750 genes are developmentally regulated. However, only about 0.3% of the Tn*5lac* insertions are in genes that are essential for development but not growth, which suggests that only about 30 of the developmentally regulated genes are essential for development. Therefore, most developmentally regulated genes are not essential for development under laboratory conditions.

The expression of some nonessential developmentally regulated genes was examined by using epistasis. The theory behind epistasis is that a strain containing a reporter gene and a mutation in the same pathway will not express the reporter if the mutation blocks the pathway first. These experiments indicate that mutations that produce morphologically different phenotypes disrupt the developmental pathway at different places (124). For example, the *asgB* mutation blocks the developmental pathway earlier than the *csgA* mutation does. Expression of many promoters was only partially inhibited by certain mutations. This result was not due to leakiness of the mutant alleles, most of which are well-characterized null mutations. Rather, it suggests that developmental genes are regulated by multiple regulatory factors. Analysis of *ops* and *tps* regulation seems to attest to the complexity of developmental gene expression (41, 113).

The collection of developmental mutants may be divided into three morphological groups: those that fail to aggregate or sporulate, those that aggregate but fail to sporulate, and those that sporulate well but aggregate poorly (167). This last class of mutants demonstrates that construction of a fruiting body is not essential for sporulation. Aberrant fruiting-body morphologies occur when the synchrony between aggregation and sporulation is lost. Myxospores are nonmotile, and fruiting-body morphogenesis necessarily ceases when sporulation is complete. This unusual arrangement of the developmental pathway may have a role in nature by ensuring that starving cells can complete sporulation even when they lack suitable resources to form a fruiting body. There appears to be a fundamental difference between *M. xanthus* and *S. aurantiaca* development in this regard. *S. aurantiaca* spore formation appears to be much more tightly coupled to fruiting-body morphogenesis and occurs only after the stalk has been completed. Disruption of aggregation results in a loss of sporulation as well (193). Analysis of the *S. aurantiaca* developmental pathway will provide an interesting contrast to the *M. xanthus* pathway.

The function of the cell-cell signaling steps may be to synchronize aggregation with sporulation both temporally and spatially. The fact that the signaling mutants are defective in both behaviors lends credence to this notion (69, 129, 209). Since the putative signaling steps regulate gene expression, they would also tend to synchronize morphological development with changes in gene expression. The six classes of *csgA* pseudorevertants have widely different phenotypes, ranging from those that sporulate only in fruiting bodies to those that sporulate more or less uniformly over the agar surface (209). Analysis of these pseudorevertants may help determine how aggregation and sporulation are synchronized.

ANALYSIS OF THE GENOME

The DNA base composition of bacteria ranges from 24 to 74 mol% G+C (177). More extreme values are theoretically unlikely for DNA molecules containing extensive protein-coding regions because of the sequence constraints imposed by the genetic code. Myxobacteria have a DNA base composition of 67 to 71 mol% G+C (107, 145, 159) and thus lie near one extreme. Owing to the redundancy of the genetic code, different codons can code for the same amino acid, and organisms with high G+C contents tend to use codons containing more G and C residues. The base composition varies with the codon position in a manner that is proportional to the overall DNA base composition for all procaryotic organisms that have been examined (9). From the data of Bibb et al. (9), an organism such as *M. xanthus*, which has 67.5 mol% G+C (163), would be expected to have G · C base pairs 69% of the time at the first codon position, 47% of the time at the second position, and 89% of the time at the third position on average. This statistic has great predictive value in examination of DNA sequences for protein-coding regions. It can be used to determine the direction of transcription, the translated reading frame, and the approximate starting point for translation (9).

Methylation of specific bases in DNA has been observed in most organisms. In procaryotes, DNA methylation is

involved in DNA replication, recombination, repair, and gene expression. In eucaryotes, methylation of cytosine residues appears to regulate gene expression and cellular differentiation (195, 264). *M. xanthus* contains 5-methylcytosine, and two-dimensional electrophoresis of DNA digested with *Msp*I and *Hpa*II was used to determine whether the methylation state of any particular cytosine residue changes during the course of development (282). The level of methylation increased as cells approached stationary phase, but only a fraction of the molecules at a particular site became methylated. Specific sequences were not methylated during the course of development. More recently it was discovered that about 15% of the adenine residues in *M. xanthus* vegetative DNA are methylated (163). Adenine methylation can serve as a positive or negative regulator of gene expression in *E. coli* (89). It remains to be determined whether adenine methylation plays any role in the regulation of *M. xanthus* development.

The sequence poly(dT-dG) · poly(dC-dA), referred to as a TG element, is widely distributed taxonomically in eucaryotic genomes. In its most common form, the thymine and guanine residues alternate for 30 to 60 bp and the TG element is capable of adopting the left-handed Z helix in vitro. The element is present in the human genome at about 50,000 copies per haploid genome (80). Although its function is unknown, potential roles in recombination and transcription regulation have been proposed. A variety of bacterial species, including *M. xanthus*, were examined for the presence of this element by hybridization, but the element was not detected in any of the archaebacteria or eubacteria examined (166).

Multicopy Single-Stranded DNA

Endogenous plasmids have not been found in *M. xanthus*, and plasmids from other gram-negative organisms, including broad-host-range plasmids in the IncP group, fail to replicate in this species (14, 221). However, *M. xanthus* does produce an unusual satellite DNA known as multicopy single-stranded DNA (msDNA) (for a review, see Lampson et al., submitted). In *M. xanthus* and *S. aurantiaca*, msDNA is present at more than 500 copies per haploid genome and is homologous to a discrete region of the chromosome (61, 62, 280). The primary and secondary structures for five different molecules (Fig. 11) are now known, and although the primary sequences are significantly different, the secondary structures are similar (38, 39, 61, 62, 98, 133, 134, 138).

M. xanthus contains two species of msDNA, one of which (Mx162) contains a ribonucleotide portion (msdRNA) composed of 77 nucleotides attached to a 162-base single-stranded DNA via a 2′-5′ phosphodiester linkage at an internal guanine ribonucleotide 20 bases from the 5′ end (38, 61, 62). The region of the genome that is homologous with msDNA has been cloned from *S. aurantiaca* (61, 62), *M. xanthus* (38), and *E. coli* (34, 40), and the DNA sequence has been determined. Primary transcripts complementary to these regions are all much larger than the msdRNA. Therefore, the proposed method of msDNA synthesis involves self-annealing of an msdRNA inverted repeat to form a primer (38); msDNA is then synthesized with reverse transcriptase by using the internal guanine ribonucleotide as the priming site. The transcript would also have to be processed by an RNase H-like enzyme. The effect of site-specific mutations on the synthesis of msDNA supports this model (91). Adjacent to the msDNA coding regions in *M. xanthus* and *E. coli* is an open reading frame whose amino acid sequence is similar to that of retrovirus reverse transcriptase (98, 134, 138). *E. coli* and *M. xanthus* strains producing msDNA have reverse transcriptase activity (133, 138). Comparison of the predicted amino acid sequence of the two *M. xanthus* proteins with that of other known reverse transcriptases of retroviruses, retrotransposons, and the *Neurospora* mitochondrial plasmid has identified a region of amino acid sequence similarity, implying a common evolutionary origin of all reverse transcriptases (98).

msDNA Mx162 from *M. xanthus* is highly conserved among more than 20 independent *M. xanthus* strains isolated from all over the world (37). msDNA has been found in the myxobacterial genera *Myxococcus*, *Stigmatella*, and *Nannocystis* and in some *Cystobacter* isolates (taxonomy is given in Table 1). Since msDNA is found on both branches of the myxobacterial tree, the ancestor of modern myxobacteria probably contained this gene. Codon usage of the two *M. xanthus* reverse transcriptase genes is typical of this species, again suggesting a long evolutionary association of these genes with myxobacteria (97, 98). Therefore, the ancestral reverse transcriptase gene probably arose in the myxobacterial lineage more than 900 million years ago, when the two myxobacterial branches diverged. msDNA and reverse transcriptase are occasionally found in some distantly related species of purple bacteria, including about 5% of the clinical isolates of *E. coli* (134). Codon usage of the *E. coli* reverse transcriptase gene is so different from that of other *E. coli* genes that the appearance of this gene in *E. coli* is likely to be much more recent than its appearance in myxobacteria (97, 98). It appears, then, that reverse transcriptase was present in the ancestor of all myxobacteria and was transferred more recently to other groups of purple bacteria.

msDNA Mx162 exists as a complex with specific proteins in the cell, and it is likely that purification of this complex will help identify additional factors involved in msDNA synthesis (261). Deletion of the gene encoding Mx162, the larger of the two msDNA molecules from *M. xanthus*, eliminated this molecule but had no detectable effect on growth, morphogenesis, or motility (36). Hence it has no detectable role in development, and its precise function remains unknown.

Genome Size

The size of the *M. xanthus* genome has been measured by renaturation kinetics (281), one- and two-dimensional agarose gel electrophoresis (281, 282), radiolabeling (286), chemical analysis (285), and pulsed-field gel electrophoresis (25). Although all estimates indicate that the genome is substantially larger than that of *E. coli*, the estimated sizes have varied from 5,690 to 12,727 kbp. In the earliest attempt to measure the genome size, Zusman and Rosenberg assayed the number of chromosomes per cell by radiolabeling DNA of exponentially growing cells for several generations, inducing myxospore formation with glycerol, and germinating the glycerol-induced myxospores in nonradioactive medium (286). From the pattern of radiolabel segregation the authors concluded that glycerol-induced myxospores contain an average of 3.3 haploid genomes per spore. The average DNA content per myxospore was estimated to be 27×10^{-9} μg by chemical analysis, and the genome size was calculated to be approximately 7,400 kbp.

Ten years later Zusman et al., using chemical and fluorescence techniques, estimated that exponentially growing cells contain 20×10^{-9} μg of DNA (285). The number of haploid

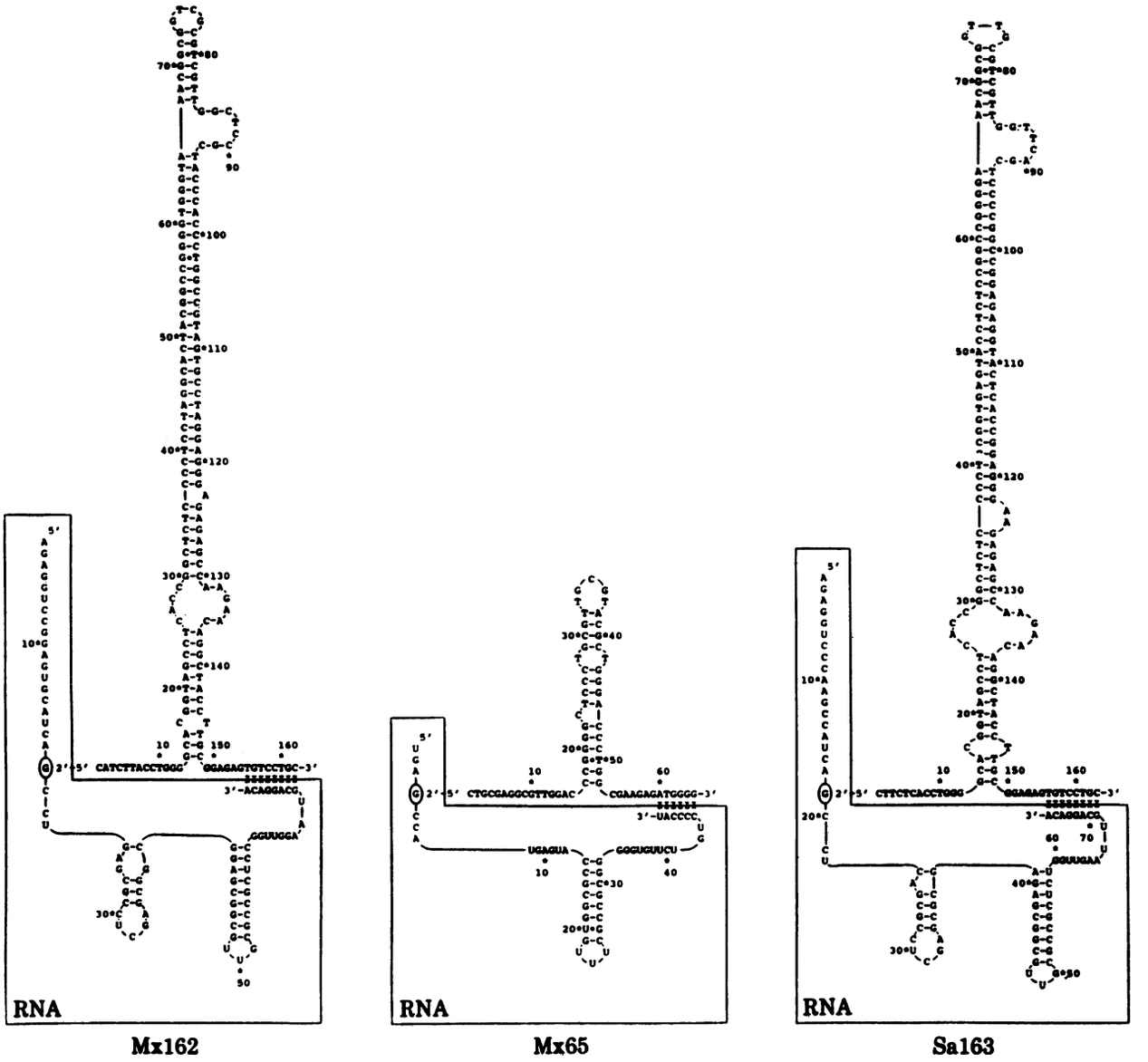
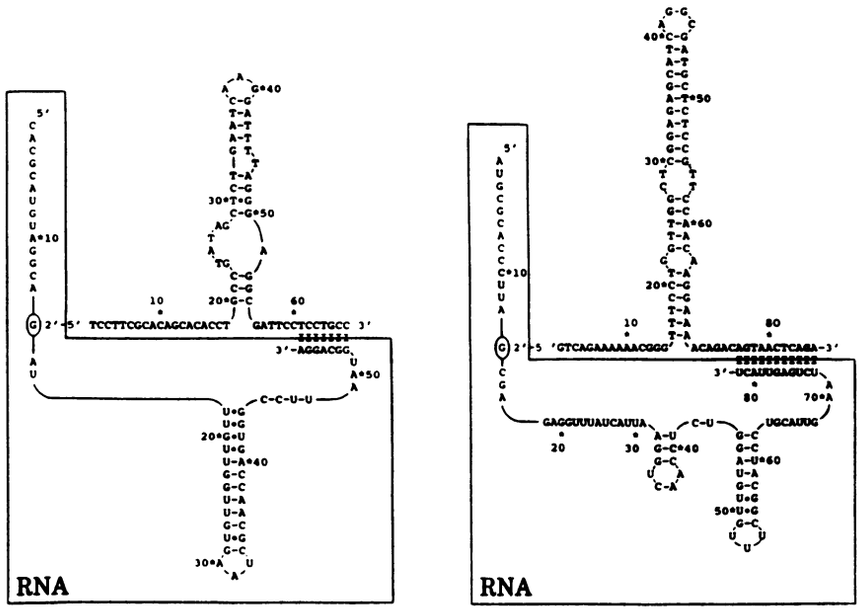


FIG. 11. Primary and secondary structures of msDNA (Lampson et al., submitted). The complete nucleotide sequence of the single-stranded DNA strand and the RNA strand (msdRNA; boxed region) of five msDNAs is shown along with the proposed secondary structure. The G residue in msdRNA containing the 2',5' branch linkage with the DNA strand is circled. Each msDNA is identified by two letters representing the genus and species of the host organism and a number corresponding to the size of the DNA in nucleotide bases. Mx162 and Mx65 are from *M. xanthus* DZF1 (38, 39); Sa163 is from *S. aurantiaca* DW4 (61, 62); Ec67 is from *E. coli* Cl-1 (134), and Ec86 is from *E. coli* B (138).

genomes per cell was determined through segregation experiments. DNA in exponentially growing cells was radioactively labeled for four generations, cells were transferred to nonradioactive medium, and the distribution of label in the cells after zero, one, and two generations followed a pattern consistent with one haploid genome per newly divided cell. A genome size of 12,727 ± 1,820 kbp was obtained, assuming that the average exponentially growing cell has 1.5 genomes.

Several years later Yee and Inouye used renaturation kinetics and one-dimensional restriction fragment analysis to estimate the size of the *M. xanthus* genome relative to that of *E. coli* (281). The genome of *M. xanthus* contains a much higher G+C composition than that of *E. coli*, and since the renaturation conditions are chosen on the basis of G+C composition of the organisms, comparable hybridization conditions are not possible. The authors suggested that the *M. xanthus* genome was 4,700 to 5,800 kbp.

Later Yee and Inouye used two-dimensional agarose gel electrophoresis to determine a genome size of about 5,700 kbp (282). Chromosomal DNA was digested with a restriction endonuclease, and the restriction fragments were separated in an agarose gel. Then this DNA was digested in situ with another enzyme, and the fragments were separated in a second dimension. The resulting two-dimensional pattern of spots contained so many overlapping restriction fragments that statistical techniques were needed to compensate for the difficulty in resolving all the restriction fragments.



Ec67 Ec86

In the most recent genome size determination, Chen et al. used pulsed-field gel electrophoresis (25). High-molecular-weight DNA was prepared in agarose and digested with the rare cutting restriction endonucleases *Ase*I and *Spe*I. The restriction fragments were separated by field inversion gel electrophoresis or contour-clamped homogeneous electric field electrophoresis. The genome size determined from the sum of the *Ase*I restriction products, 9,454 kbp, is similar to the size determined from the *Spe*I restriction products. Furthermore, 100 of 103 transposon insertions were found to be located in known *Ase*I restriction fragments, which argued that all the major restriction fragments are accounted for in this analysis. Optical renaturation kinetics placed the genome size of *S. aurantiaca* nearly identical to that of *M. xanthus* (281).

From the nucleotide sequence of the 16S and 18S rRNA molecules, all living organisms appear to be descended from a common ancestor and fall into three distinct kingdoms, archaebacteria, eubacteria, and eucaryotes (276). Genome sizes for members of these three kingdoms are compared in Fig. 12. Archaebacterial genomes have a relatively narrow size distribution, ranging from 1.6×10^6 to 4.3×10^6 bp (118, 164, 165). The eubacteria have a much wider size distribution, with several *Mycoplasma* species (196) and *Chlamydia trachomatis* (83) having genomes as small as 6×10^5 bp while heterocyst-forming cyanobacteria such as *Calothrix* species (84, 131) have genomes as large as 1.3×10^7 bp. Eucaryotic organisms have the largest range of genome sizes, extending from 1.4×10^7 to more than 1×10^{11} bp (23, 168). Myxobacterial genomes, at about 9.5×10^6 bp (25), are at the large end of the eubacterial scale and approach the size of the lower eucaryotic genomes.

The three bacterial genera with the largest genomes have complex developmental cycles involving differentiated cell types. However, it is not clear that a large genome is the result of developmental complexity. Only about 8% of the *M. xanthus* genome increases expression during development, and less than 1% of the genome, excluding housekeeping functions, is essential for development (125). Therefore, the functions of the large genome remain unknown.

A plot of the genome size frequency of 605 procaryotes shows peaks at 0.75×10^6, 1.7×10^6, and 3.9×10^6 bp (83). This result raises the possibility that larger genomes arose from smaller genomes by genome duplication (90, 242, 266). Large tandem duplications occur spontaneously with a high frequency in the enteric bacteria (1) and the myxobacteria (4). If the genome duplication hypothesis were true, one might expect to see genes with related functions located 180°

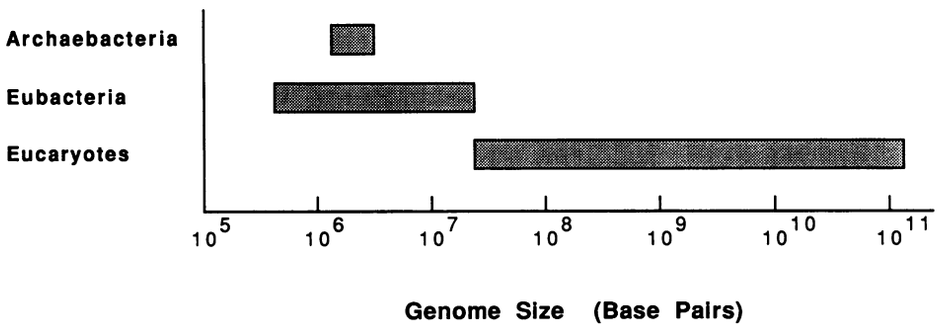


FIG. 12. Comparison of the range of genome sizes found in the three kingdoms.

from each other on the chromosome. The only *E. coli* genes with this distribution are those encoding enzymes involved in glucose metabolism (210). In *Streptomyces coelicolor*, genes controlling different steps of the same metabolic pathway are located 180° from each other and the intervening regions have no obvious functions (90).

The environmental and physiological factors constraining genome size and genome evolution are largely unknown, but a relationship with the efficiency of the respiratory system has been noted. The largest bacterial genomes are those of aerobic or photosynthetic bacteria (83). Sulfate-reducing bacteria of the genus *Desulfovibrio*, which are postulated to be descended from the anaerobic ancestor of modern myxobacteria (276), have genome sizes about one-sixth that of the myxobacteria (191). This raises the possibility that the myxobacterial genome arose from that of its anaerobic ancestors by duplication events. Replication of such a large amount of DNA would tend to place an increased metabolic burden on the cells, which could have been balanced by a bioenergetically favorable shift from anaerobic sulfate respiration, which generates low yields of ATP, to aerobic respiration.

Cell Cycle

The cell cycle of *M. xanthus* has been examined, and a comparison with the cycle of *E. coli* B/r (for a review, see reference 263) is worthwhile. When *E. coli* grows with a generation time of less than 60 min, an additional round of replication is initiated prior to the completion of the previous round of replication. Because the generation time of *M. xanthus* is always much longer than the replication time, there is no need for dichotomous replication (287). Therefore, the *M. xanthus* cell cycle is best compared with that of *E. coli* growing at generation times in excess of 90 min. In both situations there is a gap in DNA synthesis following cell division and a second gap following replication and preceding cell division (285, 287). Furthermore, the duration of the replication period increases with decreasing growth rate (28, 126, 285). However, the replication period in slow-growing *E. coli* B/r cells is about one-third of the length of the cell cycle (126), whereas the length of the replication period in *M. xanthus* is 80 to 90% of the length of the cell cycle (285). These results suggest that the cell cycle of *M. xanthus* is subject to control mechanisms that would provide an interesting contrast to those reported for enteric bacteria.

EVOLUTIONARY CONSIDERATIONS

The age of the myxobacteria can be estimated from the divergence of the 16S rRNA molecule (276). The rate of evolution of this molecule appears to have been constant throughout the evolution of the purple group of the eubacteria at 1% nucleotide substitution per 50 million years (178). Comparison of the oligonucleotide similarities of 16S rRNA from two species is often given in terms of a binary association constant, or S_{ab} value (276). The relationship between S_{ab} and percent nucleotide similarity is not precise, but can be approximated from a plot of both parameters. The minimum age of the myxobacteria can be estimated from the point at which the two major myxobacterial subgroups diverged. The S_{ab} value for the two myxobacterial subgroups is about 0.42 (140), which corresponds to a divergence at about 900 million years ago (178). The maximum age of the myxobacteria can be estimated from the S_{ab} value of the myxobacteria and their closest relatives, the bdellovibrios and the sulfate-reducing bacteria (276), which is about 0.37 (140) and corresponds to a divergence at approximately 1 billion years ago.

These data fit with the current concept of the appearance of oxygen in the Earth's atmosphere. The origin of oxygenic photosynthesis by cyanobacteria about 2.8 billion years ago was probably essential to the evolution of an aerobic atmosphere (223). Initially the free oxygen interacted with reduced inorganic materials until those materials were sufficiently oxidized to allow buildup of O_2 in the atmosphere. The O_2 concentration in the atmosphere reached 1% of present levels about 1.7 billion years ago and remained constant until about 1 billion years ago, when it began to increase again (24, 265). From 1.6 to 0.9 billion years ago there was an increase in the size, diversity, and abundance of microfossils, possibly as the consequence of the evolution of aerobic microorganisms. The earlier date corresponds to the divergence of the major groups of eubacteria (178). The purple bacteria split into their four groups about 1.1 billion years ago (178).

CONCLUSIONS

Many aspects of myxobacterial biology have no other known parallels in the bacterial world and provide an evolutionarily ancient solution to many global developmental and behavioral problems. Myxobacteria contain multigene social and adventurous behavioral systems, which are used to perform complex behaviors such as the rhythmic rippling behavior and fruiting-body morphogenesis. The developmental cycle of certain myxobacteria, such as *S. aurantiaca*, involves the specialization of cells into either stalk or spore cells, with the ultimate death of the stalk cells during fruiting-body morphogenesis. The manner in which the genetic code specifies such remarkable three-dimensional structures as the *S. aurantiaca* fruiting bodies or such synchronized cell movement as the ripples is a fundamental biological problem that is relevant to development of all higher organisms. As we approach the centennial of the discovery of the myxobacteria (255), it is clear that we still have much to learn about these fascinating creatures. However, the answers to a rich variety of important biological questions are clearly within reach.

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LITERATURE CITED

1. **Anderson, P. R., and J. R. Roth.** 1977. Tandem genetic duplications in phage and bacteria. Annu. Rev. Microbiol. **31**:473–505.
2. **Arnold, J. W., and L. J. Shimkets.** 1988. Inhibition of cell-cell interactions in *Myxococcus xanthus* by Congo red. J. Bacteriol. **170**:5765–5770.
3. **Arnold, J. W., and L. J. Shimkets.** 1988. Cell surface properties correlated with cohesion in *Myxococcus xanthus*. J. Bacteriol. **170**:5771–5777.
4. **Avery, L., and D. Kaiser.** 1983. In situ transposon replacement and isolation of a spontaneous tandem genetic duplication. Mol. Gen. Genet. **191**:99–109.
5. **Bacon, K., D. Clutter, R. H. Kottel, M. Orlowski, and D.**

White. 1975. Carbohydrate accumulation during myxospore formation in *Myxococcus xanthus*. J. Bacteriol. **124**:1635–1636.

6. **Bakker, E. P., and L. L. Randall.** 1984. The requirement for energy during the export of β-lactamase in *Escherichia coli* is fulfilled by the total proton motive force. EMBO J. **3**:895–900.
7. **Balsalobre, J. A., R. M. Ruiz-Vazquez, and F. J. Murillo.** 1987. Light induction of gene expression in *Myxococcus xanthus*. Proc. Natl. Acad. Sci. USA **84**:2359–2362.
8. **Bender, H.** 1962. Untersuchungen an *Myxococcus xanthus*. I. Bildungsbedingungen, Isolierung und Eigenschaften eines bacteriolytischen Enzymsystems. Arch. Mikrobiol. **43**:262–279.
9. **Bibb, M. J., P. R. Findlay, and M. W. Johnson.** 1984. The relationship between base composition and codon usage in bacterial genes and its use for the simple and reliable identification of protein coding regions. Gene **30**:157–166.
10. **Blackhart, B. D., and D. R. Zusman.** 1985. Cloning and complementation analysis of the "Frizzy" genes of *Myxococcus xanthus*. Mol. Gen. Genet. **198**:243–254.
11. **Blackhart, B. D., and D. R. Zusman.** 1985. "Frizzy" genes of *Myxococcus xanthus* are involved in control of the frequency of reversal of gliding motility. Proc. Natl. Acad. Sci. USA **82**:8767–8771.
12. **Blackhart, B. D., and D. R. Zusman.** 1986. Analysis of the products of the *Myxococcus xanthus frz* genes. J. Bacteriol. **166**:673–678.
13. **Breton, A. M., and J. F. Guespin-Michael.** 1987. *Escherichia coli* pH 2.5 acid phosphatase and β-lactamase TEM2 are secreted into the medium by *Myxococcus xanthus*. FEMS Microbiol. Lett. **40**:183–188.
14. **Breton, A. M., S. Jaoua, and J. Guespin-Michel.** 1985. Transfer of plasmid RP4 to *Myxococcus xanthus* and evidence for its integration into the chromosome. J. Bacteriol. **161**:523–528.
15. **Brockman, E. R., and R. L. Todd.** 1974. Fruiting myxobacters as viewed with a scanning electron microscope. Int. J. Syst. Bacteriol. **24**:118–124.
16. **Burchard, R. P.** 1970. Gliding motility mutants of *Myxococcus xanthus*. J. Bacteriol. **104**:940–947.
17. **Burchard, R. P.** 1984. Gliding motility and taxes, p. 139–161. *In* E. Rosenberg (ed.), Myxobacteria: development and cell interactions. Springer-Verlag, New York.
18. **Burchard, R. P., A. C. Burchard, and J. H. Parish.** 1977. Pigmentation phenotype instability in *Myxococcus xanthus*. Can. J. Microbiol. **23**:1657–1662.
19. **Burchard, R. P., and M. Dworkin.** 1966. Light-induced lysis and carotenogenesis in *Myxococcus xanthus*. J. Bacteriol. **91**:535–545.
20. **Burnham, J. C., S. A. Collart, and M. J. Daft.** 1984. Myxococcal predation of the cyanobacterium *Phormidium luridum* in aqueous environments. Arch. Microbiol. **137**:220–225.
21. **Burnham, J. C., S. A. Collart, and B. W. Highison.** 1981. Entrapment and lysis of the cyanobacterium *Phormidium luridum* by aqueous colonies of *Myxococcus xanthus* PCO2. Arch. Microbiol. **129**:285–294.
22. **Campos, J. M., and D. R. Zusman.** 1975. Regulation of development in *Myxococcus xanthus*: effect of 3′:5′ cyclic AMP, ADP and nutrition. Proc. Natl. Acad. Sci. USA **72**:518–522.
23. **Cavalier-Smith, T.** 1985. Eucaryote gene numbers, non-coding DNA and genome size, p. 69–103. *In* T. Cavalier-Smith (ed.), The evolution of genome size. John Wiley & Sons Ltd., Chichester, England.
24. **Chapman, D. J., and W. J. Schopf.** 1983. Biological and biochemical effects of the development of an aerobic environment, p. 302–320. *In* J. W. Schopf (ed.), Earth's earliest biosphere: its origin and evolution. Princeton University Press, Princeton, N.J.
25. **Chen, H.-W., I. M. Keseler, and L. J. Shimkets.** 1990. The genome size of *Myxococcus xanthus* determined by pulsed-field gel electrophoresis. J. Bacteriol. **172**:4206–4213.
26. **Cheng, Y., and D. Kaiser.** 1989. *dsg*, a gene required for cell-cell interaction early in *Myxococcus* development. J. Bacteriol. **171**:3719–3726.
27. **Cheng, Y., and D. Kaiser.** 1989. *dsg*, a gene required for *Myxococcus* development, is necessary for cell viability. J. Bacteriol. **171**:3727–3731.
28. **Churchwood, G., and H. Bremer.** 1977. Determination of deoxyribonucleic acid replication time in exponentially growing *Escherichia coli* B/r. J. Bacteriol. **130**:1206–1213.
29. **Colvin, J. R., and D. E. Witter.** 1983. Congo red and calcofluor white inhibition of *Acetobacter xylinum* cell growth and of bacterial cellulose microfibril formation: isolation and properties of a transient, extracellular glucan related to cellulose. Protoplasma **116**:34–70.
30. **Costerton, J. W., K.-J. Cheng, G. G. Geesey, T. I. Ladd, J. C. Nickel, M. Dasgupta, and T. J. Marrie.** 1987. Bacterial biofilms in nature and disease. Annu. Rev. Microbiol. **41**:435–464.
31. **Cumsky, M., and D. R. Zusman.** 1979. Myxobacterial hemagglutin, a development-specific lectin of *Myxococcus xanthus*. Proc. Natl. Acad. Sci. USA **76**:5505–5509.
32. **Cumsky, M., and D. R. Zusman.** 1981. Purification and characterization of myxobacterial hemagglutinin, a developmental-specific lectin of *Myxococcus xanthus*. J. Biol. Chem. **256**:12581–12588.
33. **Cumsky, M., and D. R. Zusman.** 1981. Binding properties of myxobacterial hemagglutinin. J. Biol. Chem. **256**:12596–12599.
34. **Date, T., J. M. Goodman, and W. T. Wickner.** 1980. Precoat, the precursor of M13 coat protein, requires an electrochemical potential for membrane insertion. Proc. Natl. Acad. Sci. USA **77**:4669–4673.
35. **Dawson, H., and M. V. Jones.** 1979. Cell wall turnover during myxospore formation in *Myxococcus xanthus*. J. Gen. Microbiol. **112**:143–148.
36. **Dhundale, A., T. Furuichi, M. Inouye, and S. Inouye.** 1988. Mutations that affect production of branched RNA-linked msDNA in *Myxococcus xanthus*. J. Bacteriol. **170**:5620–5624.
37. **Dhundale, A. R., T. Furuichi, S. Inouye, and M. Inouye.** 1985. Distribution of multicopy single-stranded DNA among myxobacteria and related species. J. Bacteriol. **164**:914–917.
38. **Dhundale, A., M. Inouye, and S. Inouye.** 1988. A new species of multicopy single-stranded DNA from *Myxococcus xanthus* with conserved structural features. J. Biol. Chem. **263**:9055–9058.
39. **Dhundale, A., B. Lampson, T. Furuichi, M. Inouye, and S. Inouye.** 1987. Structure of msDNA from *Myxococcus xanthus*: evidence for a long, self-annealing RNA precursor for the covalently linked, branched RNA. Cell **51**:1105–1112.
40. **Dobson, W. J., and H. D. McCurdy.** 1979. The function of fimbriae in *Myxococcus xanthus*. II. Purification and properties of *M. xanthus* fimbriae. Can. J. Microbiol. **25**:1150–1158.
41. **Downard, J.** 1987. Identification of the RNA products of the *ops* gene of *Myxococcus xanthus* and mapping of *ops* and *tps* RNAs. J. Bacteriol. **169**:1522–1528.
42. **Downard, J., and D. R. Zusman.** 1985. Differential expression of protein S genes during *Myxococcus xanthus* development. J. Bacteriol. **161**:1146–1155.
43. **Downard, J. S., S.-H. Kim, and K.-S. Kil.** 1988. Localization of the *cis*-acting regulatory DNA sequences of the *Myxococcus xanthus tps* and *ops* genes. J. Bacteriol. **170**:4931–4938.
44. **Downard, J. S., D. Kupfer, and D. R. Zusman.** 1984. Gene expression during development of *Myxococcus xanthus*: analysis of the genes for protein S. J. Mol. Biol. **175**:469–492.
45. **Dworkin, M.** 1963. Nutritional regulation of morphogenesis in *Myxococcus xanthus*. J. Bacteriol. **86**:67–72.
46. **Dworkin, M.** 1983. Tactic behavior of *Myxococcus xanthus*. J. Bacteriol. **154**:452–459.
47. **Dworkin, M.** 1985. The myxobacteria, p. 105–149. *In* M. Dworkin (ed.), Developmental biology of the bacteria. The Benjamin/Cummings Publishing Co., Inc., Menlo Park, Calif.
48. **Dworkin, M., and D. Eide.** 1983. *Myxococcus xanthus* does not respond chemotactically to moderate concentration gradients. J. Bacteriol. **154**:437–442.
49. **Dworkin, M., and S. M. Gibson.** 1964. A system for studying microbial morphogenesis: rapid formation of microcysts in *Myxococcus xanthus*. Science **146**:243–244.
50. **Dworkin, M., and D. Kaiser.** 1985. Cell interactions in myxo-

bacterial growth and development. Science **230**:18–24.
51. **Easson, D. D., Jr., A. J. Sinskey, and O. P. Peoples.** 1987. Isolation of *Zoogloea ramigera* I-16-M exopolysaccharide biosynthetic genes: evidence for instability within this region. J. Bacteriol. **169**:4518–4524.
52. **El Akoum, A., M. Vijayalakshmi, P. Cardon, B. Fournet, M. Sigot, and J. F. Guespin-Michel.** 1987. *Myxococcus xanthus* produces an extracellular glycopeptide that displays blood anticoagulant properties. Enzyme Microb. Technol. **9**:426–429.
53. **Eneguist, H. G., T. R. Hirst, S. Harayama, S. J. S. Hardy, and L. L. Randall.** 1981. Energy is required for maturation of exported proteins in *Escherichia coli*. Eur. J. Biochem. **116**: 227–233.
54. **Feldman, J. F.** 1982. Genetic approaches to circadian clocks. Annu. Rev. Plant Physiol. **33**:583–608.
55. **Filer, D., S. H. Kindler, and E. Rosenberg.** 1977. Myxospore coat synthesis in *Myxococcus xanthus*: enzymes associated with uridine 5′-diphosphate-*N*-acetylgalactosamine formation during myxospore development. J. Bacteriol. **131**:745–750.
56. **Filer, D., D. White, S. H. Kindler, and E. Rosenberg.** 1977. Myxospore coat synthesis in *Myxococcus xanthus*: in vivo incorporation of acetate and glycine. J. Bacteriol. **131**:751–758.
57. **Fink, J. S., M. Kalos, and J. F. Zissler.** 1989. Isolation of cell surface antigen mutants of *Myxococcus xanthus* by use of monoclonal antibodies. J. Bacteriol. **171**:2033–2041.
58. **Fink, J. S., and J. F. Zissler.** 1989. Characterization of lipopolysaccharide from *Myxococcus xanthus* by use of monoclonal antibodies. J. Bacteriol. **171**:2028–2032.
59. **Fink, J. S., and J. F. Zissler.** 1989. Defects in motility and development of *Myxococcus xanthus* lipopolysaccharide mutants. J. Bacteriol. **171**:2042–2048.
60. **Fowler, V. J., F. Widdel, N. Pfenning, C. R. Woese, and E. Stackebrandt.** 1986. Phylogenetic relationships of sulfate- and sulfur-reducing eubacteria. Syst. Appl. Microbiol. **8**:32–41.
61. **Furuichi, T., A. Dhundale, M. Inouye, and S. Inouye.** 1987. Branched RNA covalently linked to the 5′ end of a single-stranded DNA in *Stigmatella aurantiaca*: structure of msDNA. Cell **48**:47–53.
62. **Furuichi, T., S. Inouye, and M. Inouye.** 1987. Biosynthesis and structure of stable branched RNA covalently linked to the 5′ end of multicopy single-stranded DNA from *Stigmatella aurantiaca*. Cell **48**:55–62.
63. **Gelvan, I., M. Varon, and E. Rosenberg.** 1987. Cell-density-dependent killing of *Myxococcus xanthus* by autocide AMV. J. Bacteriol. **169**:844–848.
64. **Gibbons, N. E., P. H. A. Sneath, and S. P. Lapage.** 1984. Reference collections of bacteria—the need and requirements for type strains, p. 27–28. *In* N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore.
65. **Gill, J. S., and M. Dworkin.** 1986. Cell surface antigens during submerged development of *Myxococcus xanthus* examined with monoclonal antibodies. J. Bacteriol. **168**:505–511.
66. **Gill, J. S., and M. Dworkin.** 1988. Isolation of additional monoclonal antibodies directed against cell surface antigens of *Myxococcus xanthus* cells undergoing submerged development. J. Bacteriol. **170**:5953–5955.
67. **Gill, J. S., B. W. Jarvis, and M. Dworkin.** 1987. Inhibition of development in *Myxococcus xanthus* by monoclonal antibody 1604. Proc. Natl. Acad. Sci. USA **84**:4505–4508.
68. **Gill, R. E., and M. C. Bornemann.** 1988. Identification and characterization of the *Myxococcus xanthus bsgA* gene product. J. Bacteriol. **170**:5289–5297.
69. **Gill, R. E., and M. G. Cull.** 1986. Control of developmental gene expression by cell-to-cell interaction in *Myxococcus xanthus*. J. Bacteriol. **168**:341–347.
70. **Gill, R. E., M. G. Cull, and S. Fly.** 1988. Genetic identification and cloning of a gene required for developmental cell interactions in *Myxococcus xanthus*. J. Bacteriol. **170**:5279–5288.
71. **Gillespie, D. C., and F. D. Cook.** 1965. Extracellular enzymes from strains of *Sorangium*. Can. J. Microbiol. **11**:109–118.
72. **Gilmore, D. F., and D. White.** 1985. Energy-dependent cell cohesion in myxobacteria. J. Bacteriol. **161**:113–117.
73. **Gnosspelius, G.** 1978. Purification and properties of an extracellular protease from *Myxococcus virescens*. J. Bacteriol. **133**:17–25.
74. **Gnosspelius, G.** 1978. Myxobacterial slime and proteolytic activity. Arch. Microbiol. **116**:51–59.
75. **Grilione, P. L., and J. Pangborn.** 1975. Scanning electron microscopy of fruiting body formation by myxobacteria. J. Bacteriol. **124**:1558–1565.
76. **Hagen, D. C., A. P. Bretscher, and D. Kaiser.** 1978. Synergism between morphogenetic mutants of *Myxococcus xanthus*. Dev. Biol. **64**:284–296.
77. **Hagen, T. J., and L. J. Shimkets.** 1990. Nucleotide sequence and transcriptional products of the *csg* locus of *Myxococcus xanthus*. J. Bacteriol. **172**:15–23.
78. **Haigler, C. H., R. M. Brown, Jr., and M. Benziman.** 1980. Calcofluor white ST alters the *in vivo* assembly of cellulose microfibrils. Science **210**:903–906.
79. **Hall, J. C., and M. Rosbash.** 1988. Mutations and molecules influencing biological rhythms. Annu. Rev. Neurosci. **11**:373–393.
80. **Hamanda, H., and T. Kakunaga.** 1982. Potential Z-DNA sequences are highly dispersed in the human genome. Nature (London) **298**:396–398.
81. **Haska, G.** 1972. Extracellular lytic enzymes of *Myxococcus virescens*. III. Characterization of two endo-β-N-acetylglucasaminidases. Physiol. Plant. **27**:139–142.
82. **Haska, G., B. Noren, and G. Odham.** 1972. Effect of fatty acids on the activity of bacteriolytic enzymes. Physiol. Plant. **27**: 187–194.
83. **Herdman, M.** 1985. The evolution of bacterial genomes, p. 37–68. *In* T. Cavalier-Smith (ed.), The evolution of genome size. John Wiley & Sons, Inc., New York.
84. **Herdman, M., M. Janvier, R. Rippka, and R. Y. Stanier.** 1979. Genome size of the cyanobacteria. J. Gen. Microbiol. **111**:73–85.
85. **Herth, W.** 1980. Calcofluor white and Congo red inhibit chitin microfibril assembly of *Poterioochromas*: evidence for a gap between polymerization and microfibril formation. J. Cell Biol. **87**:442–450.
86. **Hodgkin, J., and D. Kaiser.** 1977. Cell-cell stimulation of movement in nonmotile mutants of *Myxococcus*. Proc. Natl. Acad. Sci. USA **74**:2938–2942.
87. **Hodgkin, J., and D. Kaiser.** 1979. Genetics of gliding motility in *Myxococcus xanthus* (Myxobacterales): genes controlling movements of single cells. Mol. Gen. Genet. **171**:167–176.
88. **Hodgkin, J., and D. Kaiser.** 1979. Genetics of gliding motility in *Myxococcus xanthus* (Myxobacterales): two genes systems control movement. Mol. Gen. Genet. **172**:177–191.
89. **Hoopes, B. C., and W. R. McClure.** 1987. Strategies in regulation of transcription initiation, p. 1231–1240. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
90. **Hopwood, D. A.** 1989. *Streptomyces* genetics in pure and applied research, p. 12–20. *In* C. L. Hershberger, S. W. Queener, and G. Hegeman (ed.), Genetics and molecular biology of industrial organisms. American Society for Microbiol, Washington, D.C.
91. **Hsu, M.-Y., S. Inouye, and M. Inouye.** 1989. Structural requirements of the RNA precursor for the biosynthesis of the branched RNA-linked multicopy single-stranded DNA of *Myxococcus xanthus*. J. Biol. Chem. **264**:6214–6219.
92. **Inouye, M., S. Inouye, and D. R. Zusman.** 1979. Gene expression during development of *Myxococcus xanthus*: pattern of protein synthesis. Dev. Biol. **68**:579–591.
93. **Inouye, M., S. Inouye, and D. R. Zusman.** 1979. Biosynthesis and self-assembly of protein S, a development specific protein of *Myxococcus xanthus*. Proc. Natl. Acad. Sci. USA **76**:209–213.
94. **Inouye, S.** 1990. Cloning and DNA sequence of the gene coding for the major sigma factor from *Myxococcus xanthus*. J.

Bacteriol. 172:80–85.
95. **Inouye, S., T. Franceschini, and M. Inouye.** 1983. Structural similarities between the developmental-specific protein from a gram-negative bacterium, *Myxococcus xanthus*, and calmodulin. Proc. Natl. Acad. Sci. USA **80**:6828–6833.
96. **Inouye, S., W. Harada, and M. Inouye.** 1981. Development-specific protein S of *Myxococcus xanthus*: purification and characterization. J. Bacteriol. **148**:678–683.
97. **Inouye, S., P. J. Herzer, and M. Inouye.** 1990. Two independent retrons with highly diverse reverse transcriptases in *Myxococcus xanthus*. Proc. Natl. Acad. Sci. USA **87**:942–945.
98. **Inouye, S., M.-Y. Hsu, S. Eagle, and M. Inouye.** 1989. Reverse transcriptase associated with the biosynthesis of the branched RNA-linked msDNA in *Myxococcus xanthus*. Cell **56**:709–717.
99. **Inouye, S., Y. Ike, and M. Inouye.** 1983. Tandem repeat of the genes for protein S, a development-specific protein of *Myxococcus xanthus*. J. Biol. Chem. **258**:38–40.
100. **Inouye, S., M. Inouye, B. McKeever, and R. Sarma.** 1980. Preliminary crystallographic data for protein S, a development-specific protein of *Myxococcus xanthus*. J. Biol. Chem. **255**: 3713–3714.
101. **Inouye, S., D. White, and M. Inouye.** 1980. Development of *Stigmatella aurantiaca*: effects of light and gene expression. J. Bacteriol. **141**:1360–1365.
102. **Irschick, H., R. Jansen, K. Gerthe, G. Holfe, and H. Reichenbach.** 1987. The sorangicins, novel and powerful inhibitors of eubacterial RNA polymerase isolated from myxobacteria. J. Antibiot. **40**:7–13.
103. **Janssen, G., and M. Dworkin.** 1985. Cell-cell interactions in developmental lysis of *Myxococcus xanthus*. Dev. Biol. **112**: 194–202.
104. **Jarvis, B. W., and M. Dworkin.** 1989. Purification and properties of *Myxococcus xanthus* cell surface antigen 1604. J. Bacteriol. **171**:4655–4666.
105. **Jarvis, B. W., and M. Dworkin.** 1989. Role of *Myxococcus xanthus* cell surface antigen 1604 in development. J. Bacteriol. **171**:4667–4673.
106. **Johnson, J. L.** 1984. Nucleic acids in bacterial classification, p. 8–11. *In* N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore.
107. **Johnson, J. L., and E. J. Ordal.** 1968. Deoxyribonucleic acid homology in bacterial taxonomy: effect of incubation temperature on reaction specificity. J. Bacteriol. **95**:893–900.
108. **Johnson, R. J., and D. White.** 1972. Myxospore formation in *Myxococcus xanthus*: chemical changes in the cell wall during cellular morphogenesis. J. Bacteriol. **112**:849–855.
109. **Kaiser, D.** 1979. Social gliding is correlated with the presence of pili in *Myxococcus xanthus*. Proc. Natl. Acad. Sci. USA **76**:5952–5956.
110. **Kaiser, D.** 1986. Control of multicellular development: *Dictyostelium* and *Myxococcus*. Annu. Rev. Genet. **20**:539–566.
111. **Kaiser, D.** 1989. Multicellular development in myxobacteria, p. 243–266. *In* D. A. Hopwood and K. F. Chater (ed.), Genetics of bacterial diversity. Academic Press, Inc., San Diego, Calif.
112. **Kaiser, D., and C. Crosby.** 1983. Cell movement and its coordination in swarms of *Myxococcus xanthus*. Cell Motil. **3**:227–245.
113. **Kil, K.-S., G. Brown, and J. Downard.** 1990. A segment of *Myxococcus xanthus ops* DNA functions as an upstream activation site for *tps* gene transcription. J. Bacteriol. **172**: 3081–3088.
114. **Killeen, K. P., and D. R. Nelson.** 1988. Acceleration of starvation- and glycerol-induced myxospore formation by prior heat shock in *Myxococcus xanthus*. J. Bacteriol. **170**:5200–5207.
115. **Kim, S. K., and D. Kaiser.** 1990. C-factor: a cell-cell signaling protein required for fruiting body morphogenesis of *M. xanthus*. Cell **61**:19–26.
116. **Kim, S. K., and D. Kaiser.** 1990. Purification and properties of *Myxococcus xanthus* C-factor, an intercellular signaling protein. Proc. Natl. Acad. Sci. USA **87**:3635–3639.
117. **Kim, S. K., and D. Kaiser.** 1990. Cell motility is required for the transmission of C-factor, an intercellular signal that coordinates fruiting body morphogenesis of *Myxococcus xanthus*. Genes Dev. **4**:896–905.
117a. **Kim, S. K., and D. Kaiser.** 1990. Cell alignment required in differentiation of *Myxococcus xanthus*. Science **249**:926–928.
118. **Klein, A., and M. Schnorr.** 1984. Genome complexity of methanogenic bacteria. J. Bacteriol. **158**:628–631.
119. **Kletter, B., and Y. Henis.** 1963. Comparative studies on the growth of myxobacteria on bacterial suspensions. Can. J. Microbiol. **9**:577–584.
120. **Kottle, R., and D. White.** 1974. Autolytic activity associated with myxospore formation in *Myxococcus xanthus*. Arch. Microbiol. **95**:91–95.
121. **Kottle, R. H., K. Bacon, D. Clutter, and D. White.** 1975. Coats from *Myxococcus xanthus*: characterization and synthesis during myxospore differentiation. J. Bacteriol. **124**:550–557.
122. **Kroos, L., P. Hartzell, K. Stephens, and D. Kaiser.** 1988. A link between cell movement and gene expression argues that motility is required for cell-cell signaling during fruiting body development. Genes Dev. **2**:1677–1685.
123. **Kroos, L., and D. Kaiser.** 1984. Construction of Tn*5lac*, a transposon that fuses *lacZ* expression to endogenous promoters, and its introduction into *Myxococcus xanthus*. Proc. Natl. Acad. Sci. USA **81**:5816–5820.
124. **Kroos, L., and D. Kaiser.** 1987. Expression of many developmentally regulated genes in *Myxococcus xanthus* depends on a sequence of cell interactions. Genes Dev. **1**:840–854.
125. **Kroos, L., A. Kuspa, and D. Kaiser.** 1986. A global analysis of developmentally regulated genes in *Myxococcus xanthus*. Dev. Biol. **117**:252–266.
126. **Kubitschek, H. E., and C. M. Newman.** 1978. Chromosome replication during the division cycle of slowly growing, steady-state cultures of three *Escherichia coli* B/r strains. J. Bacteriol. **136**:179–190.
127. **Kuner, J. M., and D. Kaiser.** 1982. Fruiting-body morphogenesis in submerged cultures of *Myxococcus xanthus*. J. Bacteriol. **151**:458–461.
128. **Kunze, B., N. Bedorf, W. Kohl, G. Hofle, and H. Reichenbach.** 1989. Myxochelin A, a new iron-chelating compound from *Angiococcus disciformis* (Myxobacterales): production, isolation, physico-chemical, and biological properties. J. Antibiot. **42**:14–17.
129. **Kuspa, A., and D. Kaiser.** 1989. Genes required for developmental signalling in *Myxococcus xanthus*: three *asg* loci. J. Bacteriol. **171**:2762–2772.
130. **Kuspa, A., L. Kroos, and D. Kaiser.** 1986. Intercellular signalling is required for developmental gene expression in *Myxococcus xanthus*. Dev. Biol. **117**:267–276.
131. **Lachance, M.-A.** 1981. Genetic relatedness of heterocystous cyanobacteria by deoxyribonucleic acid-deoxyribonucleic acid reassociation. Int. J. Syst. Bacteriol. **31**:139–147.
132. **Lampky, J. R.** 1976. Ultrastructure of *Polyangium cellulosum*. J. Bacteriol. **126**:1278–1284.
133. **Lampson, B. C., M. Inouye, and S. Inouye.** 1989. Reverse transcriptase with concomitant ribonuclease H activity in cell-free synthesis of branched RNA-linked msDNA of *Myxococcus xanthus*. Cell **56**:701–707.
134. **Lampson, B. C., J. Sun, M.-Y. Hsu, J. Vallejo-Ramirez, S. Inouye, and M. Inouye.** 1989. Reverse transcriptase in a clinical strain of *Escherichia coli*: production of branched RNA-linked msDNA. Science **243**:1033–1038.
135. **LaRossa, R., J. Kuner, D. Hagen, C. Manoil, and D. Kaiser.** 1983. Developmental cell interactions of *Myxococcus xanthus*: analysis of mutants. J. Bacteriol. **153**:1394–1404.
136. **Lev, M.** 1954. Demonstration of a diffusible fruiting factor in myxobacteria. Nature (London) **173**:501.
137. **Levine, H. B., S. M. Ringel, and J. M. Cobb.** 1978. Therapeutic properties of oral ambruticin (W7783) in experimental pulmonary coccidioidomycosis of mice. Chest **73**:202–206.
138. **Lim, D., and W. Maas.** 1989. Reverse transcriptase-dependent synthesis of a covalently linked, branched DNA-RNA compound in *E. coli* B. Cell **56**:891–904.
139. **Losick, R., L. Kroos, J. Errington, and P. Youngman.** 1989. Pathways of developmentally regulated gene expression in

Bacillus subtilis, p. 221–242. *In* D. A. Hopwood and K. F. Chater (ed.), Genetics of bacterial diversity. Academic Press, Inc., San Diego, Calif.

140. **Ludwig, W., K.-H. Schleifer, H. Reichenbach, and E. Stackebrandt.** 1983. A phylogenetic analysis of the myxobacteria *Myxococcus fulvus*, *Stigmatella aurantiaca*, *Cystobacter fuscus*, *Sorangium cellulosum* and *Nannocystis extendens*. Arch. Microbiol. **135**:58–62.
141. **Lunsdorf, H., and H. Reichenbach.** 1989. Ultrastructural details of the apparatus of gliding motility of *Myxococcus fulvus* (Myxobacterales). J. Gen. Microbiol. **135**:1633–1641.
142. **MacRae, T. H., W. J. Dobson, and H. W. McCurdy.** 1977. Fimbriation in gliding bacteria. Can. J. Microbiol. **23**:1096–1108.
143. **Maeba, P. Y.** 1983. Iodination of *Myxococcus xanthus* during development. J. Bacteriol. **155**:1033–1041.
144. **Maeba, P. Y.** 1986. Isolation of a surface glycoprotein from *Myxococcus xanthus*. J. Bacteriol. **166**:644–650.
145. **Mandel, M., and E. R. Leadbetter.** 1965. Deoxyribonucleic acid base composition of myxobacteria. J. Bacteriol. **90**:1795–1796.
146. **Manoil, C., and D. Kaiser.** 1980. Accumulation of guanosine tetraphosphate and guanosine pentaphosphate in *Myxococcus xanthus* during starvation and myxospore formation. J. Bacteriol. **141**:297–304.
147. **Manoil, C., and D. Kaiser.** 1980. Guanosine pentaphosphate and guanosine tetraphosphate accumulation and induction of *Myxococcus xanthus* fruiting-body development. J. Bacteriol. **141**:305–315.
148. **Manoil, C., and D. Kaiser.** 1980. Purine-containing compounds, including cyclic adenosine 3′,5′-monophosphate, induce fruiting of *Myxococcus xanthus* by nutritional imbalance. J. Bacteriol. **141**:374–377.
149. **Margalith, P.** 1962. Bacteriolytic principles of *Myxococcus fulvus*. Nature (London) **196**:1335–1336.
150. **Martinez-Laborda, A., M. Elias, R. Ruiz-Vazquez, and F. J. Murillo.** 1986. Insertion of Tn*5* linked to mutations affecting carotenoid synthesis in *Myxococcus xanthus*. Mol. Gen. Genet. **205**:107–114.
151. **Martinez-Laborda, A., and F. J. Murillo.** 1989. Genic and alleleic interactions in the carotenogenic response of *Myxococcus xanthus* to blue light. Genetics **122**:481–490.
152. **Masson, P. J., and J. F. Guespin-Michel.** 1988. An extracellular blood-anticoagulant glycopeptide produced exclusively during vegetative growth by *Myxococcus xanthus* and other myxobacteria is not co-regulated with other extracellular macromolecules. J. Gen. Microbiol. **134**:801–806.
153. **Mayo, K. A., and D. Kaiser.** 1989. asgB, a gene required early for developmental signalling, aggregation, and sporulation of *Myxococcus xanthus*. Mol. Gen. Genet. **218**:409–418.
154. **McBride, M. J., R. A. Weinberg, and D. R. Zusman.** 1989. "Frizzy" aggregation genes of the gliding bacterium *Myxococcus xanthus* show sequence similarities to the chemotaxis genes of enteric bacteria. Proc. Natl. Acad. Sci. USA **86**:424–428.
155. **McBride, M. J., and D. R. Zusman.** 1989. Trehalose accumulation in vegetative cells and spores of *Myxococcus xanthus*. J. Bacteriol. **171**:6383–6386.
156. **McCleary, W. R., M. J. McBride, and D. R. Zusman.** 1990. Developmental sensory transduction in *Myxococcus xanthus* involves methylation and demethylation of FrzCD. J. Bacteriol. **172**:4877–4887.

156a. **McCleary, W. R., and D. R. Zusman.** 1990. FrzE of *Myxococcus xanthus* is homologous to both CheA and CheY of *Salmonella typhimurium*. Proc. Natl. Acad. Sci. USA **87**:5898–5902.

157. **McCurdy, H. D.** 1969. Studies on the taxonomy of the Myxobacterales. I. Record of Canadian isolates and survey methods. Can. J. Microbiol. **15**:1453–1461.
158. **McCurdy, H. D.** 1989. Fruiting gliding bacteria: the myxobacteria, p. 2139–2168. *In* J. T. Staley, M. P. Bryant, N. Pfennig, and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 3. The Williams & Wilkins Co., Baltimore.
159. **McCurdy, H. D., and S. Wolf.** 1967. Deoxyribonucleic acid base composition of fruiting Myxobacterales. Can. J. Microbiol. **13**:1707–1708.
160. **McNab, R. M.** 1987. Motility and chemotaxis, p. 732–759. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
161. **McVittie, A., F. Messik, and S. A. Zahler.** 1962. Developmental biology of *Myxococcus*. J. Bacteriol. **84**:546–551.
162. **McVittie, A., and S. A. Zahler.** 1962. Chemotaxis in *Myxococcus*. Nature (London) **194**:1299–1300.
163. **Mesbah, M., U. Premachandran, and W. B. Whitman.** 1989. Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. Int. J. Syst. Bacteriol. **39**:159–167.
164. **Mitchell, R. M., L. A. Loeblich, and L. C. Klotz.** 1979. DNA organization of *Methanobacterium thermoautotrophicum*. Science **204**:1082–1084.
165. **Moore, R. L., and B. J. McCarthy.** 1969. Base sequence homology and renaturation studies of the deoxyribonucleic acid of extremely halophilic bacteria. J. Bacteriol. **99**:255–262.
166. **Morris, J., S. R. Kushner, and R. Ivarie.** 1986. The simple repeat poly(dT-dG) · poly(dC-dA) common to eucaryotes is absent from eubacteria and archaebacteria and rare in protozoans. Mol. Biol. Evol. **3**:343–355.
167. **Morrison, C. E., and D. R. Zusman.** 1979. *Myxococcus xanthus* mutants with temperature-sensitive, stage-specific defects: evidence for independent pathways in development. J. Bacteriol. **140**:1036–1042.
168. **Mortimer, R. K., and D. Schild.** 1985. Genetic map of *Saccharomyces cerevisiae*, edition 9. Microbiol. Rev. **49**:181–212.
169. **Nelson, D. R., M. G. Cumsky, and D. R. Zusman.** 1981. Localization of myxobacterial hemagglutinin in the periplasmic space and on the cell surface of *Myxococcus xanthus* during developmental aggregation. J. Biol. Chem. **256**:12589–12595.
170. **Nelson, D. R., and K. P. Killeen.** 1986. Heat shock proteins of vegetative and fruiting *Myxococcus xanthus* cells. J. Bacteriol. **168**:1100–1106.
171. **Nelson, D. R., and D. R. Zusman.** 1983. Evidence for long-lived mRNA during fruiting body formation in *Myxococcus xanthus*. Proc. Natl. Acad. Sci. USA **80**:1467–1471.
172. **Nelson, D. R., and D. R. Zusman.** 1983. Transport and localization of protein S, a spore coat protein, during fruiting-body formation by *Myxococcus xanthus*. J. Bacteriol. **154**:547–553.
173. **Newton, A.** 1989. Differentiation in *Caulobacter*: flagellum development, mobility and chemotaxis, p. 199–220. *In* D. A. Hopwood and K. F. Chater (ed.), Genetics of bacterial diversity. Academic Press, Inc., San Diego, Calif.
174. **Nicaud, J., A. Breton, G. Younes, and J. Guespin-Michel.** 1984. Mutants of *Myxococcus xanthus* impaired in protein secretion: an approach to the study of a secretory mechanism. Appl. Microbiol. Biotechnol. **20**:344–350.
175. **Noren, B.** 1960. Notes on the bacteriolytic activity of *Myxococcus virescens*. Sven. Bot. Tidskr. **54**:550–560.
176. **Noren, B.** 1960. Lytic activity on autoclaved and on intact eubacterial cells by a preparation U2D, obtained from a metabolic solution of *Myxococcus virescens*. Bataniska Notiser **113**:320–336.
177. **Normore, W. M.** 1976. Guanine-plus-cytosine (GC) composition of the DNA of bacteria, fungi, algae, and protozoa, p. 65–235. *In* G. D. Fasman (ed.), CRC handbook of biochemistry and molecular biology, vol. II, 3rd ed. CRC Press, Inc., Cleveland, Ohio.
178. **Ochman, H., and A. C. Wilson.** 1987. Evolution in bacteria: evidence for a universal substitution rate in cellular genomes. J. Mol. Evol. **26**:74–86.
179. **O'Connor, K. A., and D. R. Zusman.** 1983. Coliphage P1-mediated transduction of cloned DNA from *Escherichia coli* to *Myxococcus xanthus*: use for complementation and recombinational analysis. J. Bacteriol. **155**:317–329.
180. **O'Connor, K. A., and D. R. Zusman.** 1988. Reexamination of the role of autolysis in the development of *Myxococcus xanthus*. J. Bacteriol. **170**:4103–4112.
181. **O'Connor, K. A., and D. R. Zusman.** 1989. Patterns of cellular

interactions during fruiting-body formation in *Myxococcus xanthus*. J. Bacteriol. **171**:6013–6024.
182. **O'Connor, K. A., and D. R. Zusman.** 1990. Genetic analysis of the *tag* mutants of *Myxococcus xanthus* provides evidence for two developmental aggregation systems. J. Bacteriol. **172**: 3868–3878.
183. **Orlowski, M., P. Martin, D. White, and M. C. Wong.** 1972. Changes in activity of glyoxylate cycle enzymes during myxospore development in *Myxococcus xanthus*. J. Bacteriol. **111**: 784–790.
184. **Orndorff, P. E., and M. Dworkin.** 1980. Separation and properties of the cytoplasmic and outer membranes of vegetative cells of *Myxococcus xanthus*. J. Bacteriol. **141**:914–927.
185. **Orndorff, P. E., and M. Dworkin.** 1982. Synthesis of several membrane proteins during developmental aggregation in *Myxococcus xanthus*. J. Bacteriol. **149**:29–39.
186. **Oxford, A. E.** 1947. Observations concerning the growth and metabolic activities of myxococci in simple protein-free medium. J. Bacteriol. **53**:129–138.
187. **Oyaizu, H., and C. R. Woese.** 1985. Phylogenetic relationships among the sulfate respiring bacteria, myxobacteria and purple bacteria. Syst. Appl. Microbiol. **6**:257–263.
188. **Panasenko, S. M.** 1985. Methylation of macromolecules during development in *Myxococcus xanthus*. J. Bacteriol. **164**:495–500.
189. **Panasenko, S. M., B. Jann, and K. Jann.** 1989. Novel changes in the carbohydrate portion of *Myxococcus xanthus* lipopolysaccharide during development. J. Bacteriol. **171**:1835–1840.
190. **Pittendrigh, C. S.** 1954. On temperature independence in the clock-system controlling emergence in *Drosophila*. Proc. Natl. Acad. Sci. USA **40**:1018–1029.
191. **Postgate, J. R., H. M. Kent, R. L. Robson, and J. A. Chesshyre.** 1984. The genomes of *Desulfovibrio gigas* and *D. vulgaris*. J. Gen. Microbiol. **130**:1597–1601.
192. **Qualls, G. T., K. Stephens, and D. White.** 1978. Morphogenetic movements and multicellular development in the fruiting myxobacterium *Stigmatella aurantiaca*. Dev. Biol. **66**:270–274.
193. **Qualls, G. T., K. Stephens, and D. White.** 1978. Light-stimulated morphogenesis in the fruiting myxobacterium *Stigmatella aurantiaca*. Science **201**:444–445.
194. **Qualls, G. T., and D. White.** 1982. Developmental cell cohesion in *Stigmatella aurantiaca*. Arch. Microbiol. **131**:334–337.
195. **Razin, A., and A. D. Riggs.** 1980. DNA methylation and gene function. Science **210**:6094–6100.
196. **Razin, S., M. F. Barile, R. Harasawa, D. Amikam, and G. Glaser.** 1983. Characterization of the *Mycoplasma* genome. Yale J. Biol. Med. **56**:357–366.
197. **Reichenbach, H.** 1965. Rhythmische Vorange bei der Schwarmentfaltung von Myxobacterien. Ber. Dtsch. Bot. Ges. **78**: 102–105.
198. **Reichenbach, H.** 1966. *Myxococcus* spp. (Myxobacterales) Schwarmentwicklung und Bildung von Protocysten, p. 557–578. *In* G. Wolf (ed.), Encyclopedia Cinematographica, film E778/1965. Institut für den Wissenschaftlichen Film, Göttingen, Federal Republic of Germany.
199. **Reichenbach, H.** 1986. The myxobacteria: common organisms with an uncommon behavior. Microbiol. Sci. **3**:268–274.
200. **Reichenbach, H., and M. Dworkin.** 1970. Induction of myxospore formation in *Stigmatella aurantiaca* (*Myxobacterales*) by monovalent cations. J. Bacteriol. **101**:325–326.
201. **Reichenbach, H., and M. Dworkin.** 1981. The order Myxobacterales, p. 328–355. *In* M. P. Starr, H. Stolp, H. G. Trüper, A. Balows, and H. G. Schlegel (ed.), The prokaryotes: a handbook on habitats, isolation, and identification of bacteria. Springer-Verlag KG, Berlin.
202. **Reichenbach, H., H. K. Galle, and H. H. Heunert.** 1975–1976. *Stigmatella aurantiaca* spp. (Myxobacterales) Schwarmentwicklung und Fruchtkorperbildung. Encyclopedia Cinematographica, film E2421. Institut für den Wissenschaftlichen Film, Göttingen, Federal Republic of Germany.
203. **Reichenbach, H., K. Gerth, H. Irschik, B. Kunze, and G. Holfe.** 1988. Myxobacteria: a new source of antibiotics. Biotechnology **6**:115–121.
204. **Reichenbach, H., H. H. Heunert, and H. Kuczka.** 1965. Schwarmentwicklung und Morphogenese bei Myxobakterien—*Archangium*, *Myxococcus*, *Chondrococcus*, and *Chondromyces*. Encyclopedia Cinematographica, film C893. Institut für den Wissenschaftlichen Film, Göttingen, Federal Republic of Germany.
205. **Reichenbach, H., H. H. Heunert, and H. Kuczka.** 1965. *Chondromyces apiculatus* (Myxobacterales)—Schwarmentwicklung und Morphogenese. Encyclopedia Cinematographica, film E779. Institut für den Wissenschaftlichen Film, Göttingen, Federal Republic of Germany.
206. **Reichenbach, H., H. H. Heunert, and H. Kuczka.** 1965. *Myxococcus* spp. (Myxobacterales)—Schwarmentwicklung und Bildung von Protocysten. Encyclopedia Cinematographica, film E778. Institut für den Wissenschaftlichen Film, Göttingen, Federal Republic of Germany.
207. **Reichenbach, H., H. H. Heunert, and H. Kuczka.** 1965. *Archangium violaceum* (Myxobacterales)—Schwarmentwicklung und Bildung von Protocysten. Encyclopedia Cinematographica, film E777. Institut für den Wissenschaftlichen Film, Göttingen, Federal Republic of Germany.
208. **Reichenbach, H., and H. Kleinig.** 1984. Pigments of myxobacteria, p. 127–137. *In* E. Rosenberg (ed.), Myxobacteria: development and cell interactions. Springer-Verlag, New York.
209. **Rhie, H. G., and L. J. Shimkets.** 1989. Developmental bypass suppression of *Myxococcus xanthus csgA* mutations. J. Bacteriol. **71**:3268–3276.
210. **Riley, M., L. Solomon, and D. Zipkas.** 1978. Relationship between gene function and gene location in *Escherichia coli*. J. Mol. Evol. **11**:47–56.
211. **Ringel, S. M., R. C. Greenough, S. Roemer, D. Conner, A. L. Gutt, B. Blair, G. Kanter, and M. von Strandtmann.** 1977. Ambruticin (W7783), a new antifungal antibiotic. J. Antibiot. **30**:371–375.
212. **Romeo, J. M., B. Esmon, and D. R. Zusman.** 1986. Nucleotide sequence of the myxobacterial hemagglutin gene contains four homologous domains. Proc. Natl. Acad. Sci. USA **83**:6332–6336.
213. **Romeo, J. M., and D. R. Zusman.** 1987. Cloning of the gene for myxobacterial hemagglutin and isolation and analysis of structural gene mutations. J. Bacteriol. **169**:3801–3808.
214. **Roper, M. M., and K. C. Marshall.** 1978. Biological control agents of sewage bacteria in marine habitats. Aust. J. Mar. Freshwater Res. **29**:335–343.
215. **Rosenberg, E. (ed.).** 1984. Myxobacteria: development and cell interactions. Springer-Verlag, New York.
216. **Rosenberg, E., K. H. Keller, and M. Dworkin.** 1977. Cell density-dependent growth of *Myxococcus xanthus* on casein. J. Bacteriol. **129**:770–777.
217. **Rosenberg, E., and M. Varon.** 1984. Antibiotics and lytic enzymes, p. 109–125. *In* E. Rosenberg (ed.), Myxobacteria: development and cell interactions, Springer-Verlag, New York.
218. **Rosenbluh, A., R. Nir, E. Sahar, and E. Rosenberg.** 1989. Cell-density-dependent lysis and sporulation of *Myxococcus xanthus* in agarose microbeads. J. Bacteriol. **171**:4923–4929.
219. **Rosenbluh, A., and E. Rosenberg.** 1989. Autocide AMI rescues development in *dsg* mutants of *Myxococcus xanthus*. J. Bacteriol. **171**:1513–1518.
220. **Rosenbluh, A., and E. Rosenberg.** 1989. Sporulation of *Myxococcus xanthus* in liquid shake flask cultures. J. Bacteriol. **171**:4521–4524.
221. **Saulnier, P., J. Hanquier, S. Jaoua, H. Reichenbach, and J. F. Guespin-Michel.** 1988. Utilization of IncP-1 plasmids as vectors for transposon mutagenesis in myxobacteria. J. Gen. Microbiol. **134**:2889–2895.
222. **Schnepf, E., G. Deichgraber, and W. Herth.** 1982. Development of cell wall appendages in *Acanthosphaera zachariasi* (Chorococcales): kinetics, site of cellulose synthesis and of microfibril assembly, and barb formation. Protoplasma **110**:203–214.
223. **Schopf, J. W., J. M. Hayes, and M. R. Walter.** 1983. Evolution of earth's earliest ecosystems: recent progress and unsolved problems, p. 361–384. *In* J. W. Schopf (ed.), Earth's earliest

biosphere: its origin and evolution. Princeton University Press, Princeton, N.J.
224. **Scott, M. P., and P. H. O'Farrell.** 1986. Spatial programming of gene expression in early *Drosophila* embryogenesis. Annu. Rev. Cell Biol. **2**:49–80.
225. **Shilo, M.** 1970. Lysis of blue-green algae by myxobacter. J. Bacteriol. **104**:453–461.
226. **Shimkets, L. J.** 1984. Nutrition, metabolism, and the initiation of development, p. 91–107. *In* E. Rosenberg (ed.), Myxobacteria: development and cell interactions. Springer-Verlag, New York.
227. **Shimkets, L. J.** 1986. Correlation of energy-dependent cell cohesion with social motility in *Myxococcus xanthus*. J. Bacteriol. **166**:837–841.
228. **Shimkets, L. J.** 1986. Role of cell cohesion in *Myxococcus xanthus* fruiting-body formation. J. Bacteriol. **166**:842–848.
229. **Shimkets, L. J.** 1987. Control of morphogenesis in myxobacteria. Crit. Rev. Microbiol. **14**:195–227.
230. **Shimkets, L. J.** 1989. The role of the cell surface in the social and adventurous behavior of the myxobacteria. Mol. Microbiol. **3**:1295–1298.
231. **Shimkets, L. J., and S. J. Asher.** 1988. Use of recombination techniques to examine the structure of the *csg* locus of *Myxococcus xanthus*. Mol. Gen. Genet. **211**:63–71.
232. **Shimkets, L. J., and M. Dworkin.** 1981. Excreted adenosine is a cell density signal for the initiation of fruiting body formation in *Myxococcus xanthus*. Dev. Biol. **84**:51–60.
233. **Shimkets L. J., R. E. Gill, and D. Kaiser.** 1983. Developmental cell interactions in *Myxococcus xanthus* and the *spoC* locus. Proc. Natl. Acad. Sci. USA **80**:1406–1410.
234. **Shimkets, L. J., and D. Kaiser.** 1982. Induction of coordinated cell movement in *Myxococcus xanthus*. J. Bacteriol. **152**:451–461.
235. **Shimkets, L. J., and D. Kaiser.** 1982. Murein components rescue developmental sporulation of *Myxococcus xanthus*. J. Bacteriol. **152**:462–470.
236. **Shimkets, L. J., and H. Rafiee.** 1990. CsgA, an extracellular protein essential for *Myxococcus xanthus* development. J. Bacteriol. **172**:5299–5306.
237. **Shimkets, L. J., and T. W. Seale.** 1974. Fruiting-body formation and myxospore differentiation and germination in *Myxococcus xanthus* viewed by scanning electron microscopy. J. Bacteriol. **121**:711–720.
238. **Singh, B. N.** 1947. Myxobacteria in soils and composts: their distribution, number, and lytic action on bacteria. J. Gen. Microbiol. **1**:1–10.
239. **Smit, G., J. W. Kilne, and B. J. J. Lutgenberg.** 1987. Involvement of both cellulose fibrils and a Ca^{2+}-dependent adhesin in the attachment of *Rhizobium leguminosarum* to pea root hair tips. J. Bacteriol. **169**:4294–4301.
240. **Smith, C. L., J. G. Econome, A. Schutt, S. Klco, and C. R. Cantor.** 1987. A physical map of the *Escherichia coli* K12 genome. Science **236**:1448–1453.
241. **Sodergren, E., and D. Kaiser.** 1983. Insertions of Tn*5* near genes that govern stimulatable cell motility in *Myxococcus*. J. Mol. Biol. **43**:28–29.
242. **Stahl, F. W.** 1967. Circular genetic maps. J. Cell. Physiol. **70**(Suppl. 1):1–12.
243. **Stanier, R. Y.** 1942. A note on elacticotaxis in myxobacteria. J. Bacteriol. **44**:405–412.
244. **Stephens, K., P. Hartzell, and D. Kaiser.** 1989. Gliding motility in *Myxococcus xanthus*: *mgl* locus, RNA, and predicted protein products. J. Bacteriol. **171**:819–830.
245. **Stephens, K., G. D. Hegeman, and D. White.** 1982. Pheromone produced by the myxobacterium *Stigmatella aurantiaca*. J. Bacteriol. **149**:739–747.
246. **Stephens, K., and D. Kaiser.** 1987. Genetics of gliding motility in *Myxococcus xanthus*: molecular cloning of the *mgl* locus. Mol. Gen. Genet. **207**:256–266.
247. **Stephens, K., and D. White.** 1980. Morphogenetic effects of light and guanine derivatives on the fruiting myxobacterium *Stigmatella aurantiaca*. J. Bacteriol. **144**:322–326.
248. **Sudo, S. Z., and M. Dworkin.** 1969. Resistance of vegetative cells and microcysts of *Myxococcus xanthus*. J. Bacteriol. **98**:883–887.
249. **Sudo, S. Z., and M. Dworkin.** 1972. Bacteriolytic enzymes produced by *Myxococcus xanthus*. J. Bacteriol. **110**:236–245.
250. **Sulston, J. E., and H. R. Horvitz.** 1977. Post-embryonic cell lineages of the nemetode, *Caenorhabditis elegans*. Dev. Biol. **56**:110–156.
251. **Sutherland, I. W., and S. Thomson.** 1975. Comparison of the polysaccharides produced by *Myxococcus* strains. J. Gen. Microbiol. **89**:124–132.
252. **Teintze, M., T. Furuichi, R. Thomas, M. Inouye, and S. Inouye.** 1985. Differential expression of two homologous genes coding for spore-specific proteins in *Myxococcus xanthus*, p. 253–260. *In* J. Hoch and P. Setlow (ed.), Molecular biology of microbial differentiation. American Society for Microbiology, Washington, D.C.
253. **Teintze, M., M. Inouye, and S. Inouye.** 1988. Characterization of calcium-binding sites in development-specific protein S of *Myxococcus xanthus* using site-specific mutagenesis. J. Biol. Chem. **263**:1199–1203.
254. **Teintze, M., R. Thomas, T. Furuichi, M. Inouye, and S. Inouye.** 1985. Two homologous genes coding for spore-specific proteins are expressed at different times during development of *Myxococcus xanthus*. J. Bacteriol. **163**:121–125.
255. **Thaxter, R.** 1892. On the Myxobacteriaceae, a new order of Schizomycetes. Bot. Gaz. **17**:389–406.
256. **Tomchik, K. J., and P. N. Devreotes.** 1981. Adenosine 3′,5′-monophosphate waves in Dictyostelium discoideum: a demonstration by isotope dilution-fluorography. Science **212**:443–446.
257. **Torti, S., and D. Zusman.** 1981. Genetic characterization of aggregation-defective developmental mutants of *Myxococcus xanthus*. J. Bacteriol. **147**:768–775.
258. **Varon, M., S. Cohen, and E. Rosenberg.** 1984. Autocides produced by *Myxococcus xanthus*. J. Bacteriol. **167**:356–361.
259. **Varon, M., A. Tietz, and E. Rosenberg.** 1986. *Myxococcus xanthus* autocide AMI. J. Bacteriol. **167**:356–361.
260. **Vasquez, G. M., F. Qualls, and D. White.** 1985. Morphogenesis of *Stigmatella aurantiaca* fruiting bodies. J. Bacteriol. **163**: 515–521.
261. **Viswanathan, M., M. Inouye, and S. Inouye.** 1989. *Myxococcus xanthus* msDNA · Mx162 exists as a complex with proteins. J. Biol. Chem. **264**:13665–13671.
262. **Voelz, H., and H. Reichenbach.** 1969. Fine structure of fruiting bodies of *Stigmatella aurantiaca* (*Myxobacterales*). J. Bacteriol. **99**:856–866.
263. **von Meyenberg, K., and F. G. Hansen.** 1987. Regulation of chromosome replication, p. 1555–1577. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
264. **Waalwijk, C., and R. A. Flavell.** 1978. DNA methylation at a CCGG sequence in the large intron of the rabbit β-globin gene: tissue specific variations. Nucleic Acids Res. **5**:4631–4641.
265. **Walker, J. C., G. C. Klein, M. Schidlowski, J. W. Schopf, D. J. Stevenson, and M. R. Walter.** 1983. Environmental evolution of the archean-early proterozoic earth, p. 260–290. *In* J. W. Schopf (ed.), Earth's earliest biosphere: its origin and evolution. Princeton University Press, Princeton, N.J.
266. **Wallace, D. C., and H. J. Morowitz.** 1973. Genome size and evolution. Chromosoma **40**:121–126.
267. **Wayne, L. G., D. J. Brenner, R. R. Colwell, P. A. D. Grimont, O. Kandler, M. I. Krichevsky, L. H. Moore, W. E. C. Moore, R. G. E. Murray, E. Stackebrandt, M. P. Starr, and H. G. Trüper.** 1987. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. Int. J. Syst. Bacteriol. **37**:463–464.
268. **Weinberg, R. A., and D. R. Zusman.** 1989. Evidence that the *Myxococcus xanthus frz* genes are developmentally regulated. J. Bacteriol. **171**:6174–6186.
269. **Weinberg, R. A., and D. R. Zusman.** 1990. Alkaline, acid, and neutral phosphatase activities are induced during development in *Myxococcus xanthus*. J. Bacteriol. **172**:2294–2302.
270. **White, D.** 1981. Cell interactions and control of development in

myxobacteria populations. Int. Rev. Cytol. **72**:203–227.
271. **White, D., M. Dworkin, and D. J. Tipper.** 1968. Peptidoglycan of *Myxococcus xanthus*: structure and relation to morphogenesis. J. Bacteriol. **95**:2186–2197.
272. **White, D., W. Shropshire, Jr., and K. Stephens.** 1980. Photocontrol of development by *Stigmatella aurantiaca*. J. Bacteriol. **142**:1023–1024.
273. **Wireman, J. W.** 1979. Developmental induction of *Myxococcus xanthus* myxospores. J. Bacteriol. **140**:147–153.
274. **Wireman, J. W., and M. Dworkin.** 1975. Morphogenesis and developmental interactions in myxobacteria. Science **189**:516–522.
275. **Wireman, J. W., and M. Dworkin.** 1977. Developmentally induced autolysis during fruiting-body formation by *Myxococcus xanthus*. J. Bacteriol. **129**:796–802.
276. **Woese, C. R.** 1987. Bacterial evolution. Microbiol. Rev. **51**: 221–271.
277. **Womack, B. J., D. F. Gilmore, and D. White.** 1989. Calcium requirement for gliding motility in myxobacteria. J. Bacteriol. **171**:6093–6096.
278. **Wood, P. J.** 1980. Specificity in the interaction of direct dyes with polysaccharides. Carbohydr. Res. **81**:271–287.
279. **Woodward, D. O., and M. L. Sargent.** 1973. Circadian rhythms in *Neurospora*, p. 282–296. *In* A. Perez-Miravete (ed.), Behavior of microorganisms. Plenum Publishing Corp., New York.
280. **Yee, T., T. Furuichi, S. Inouye, and M. Inouye.** 1984. Multicopy single-stranded DNA isolated from a gram-negative bacterium, *Myxococcus xanthus*. Cell **38**:203–209.
281. **Yee, T., and M. Inouye.** 1981. Reexamination of the genome size of myxobacteria, including the use of a new method for genome size analysis. J. Bacteriol. **145**:1257–1265.
282. **Yee, T., and M. Inouye.** 1982. Two-dimensional DNA electrophoresis applied to the study of DNA methylation and the analysis of genome size in *Myxococcus xanthus*. J. Mol. Biol. **154**:181–196.
283. **Zusman, D. R.** 1982. "Frizzy" mutants: a new class of aggregation-defective developmental mutants of *Myxococcus xanthus*. J. Bacteriol. **150**:1430–1437.
284. **Zusman, D. R.** 1984. Cell-cell interactions and development in *Myxococcus xanthus*. Q. Rev. Biol. **59**:119–138.
285. **Zusman, D. R., D. M. Krotoski, and M. Cumsky.** 1978. Chromosome replication in *Myxococcus xanthus*. J. Bacteriol. **133**:122–129.
286. **Zusman, D. R., and E. Rosenberg.** 1968. Deoxyribonucleic acid synthesis during microcyst germination in *Myxococcus xanthus*. J. Bacteriol. **96**:981–986.
287. **Zusman, D. R., and E. Rosenberg.** 1970. DNA cycle of *Myxococcus xanthus*. J. Mol. Biol. **49**:609–619.